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Procedures used by a number of laboratories for the analysis of asbestos fibers in water samples were investigated using standardized aqueous dispersions of chrysotile, crocidolite, and taconite tailings. Filtration problems when using polycarbonate filters were solved, and an optimum filtration technique was developed that permits microscopically uniform deposits to be obtained. Replicate analyses established fiber losses and reproducibility of five filter preparation techniques. The uniformity of the deposits on all the final electron microscope preparations was expressed quantitatively, and the most satisfactory performance was given by all of the Nuclepore techniques. Ultrasonic treatment at 20 kHz increased fiber concentrations of chrysotile suspensions but had no effect on crocidolite suspensions.

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PREPARATION OF WATER SAMPLES FOR ASBESTOS FIBER COUNTING BY ELECTRON MICROSCOPY

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Analytical Chemistry Branch develops and assesses new techniques for identifying and measuring chemical constituents of water and soil.

The widespread use of asbestos-containing materials gives rise to concern about exposure of the general population to low level concentrations in air, water supplies, and food. Although hazards associated with the inhalation of asbestos at high concentrations are recognized, the health significance of ingested particles is not fully understood. An important first step is the development of a method for quantitative determination of trace concentrations of asbestos minerals in water. This report, which investigates several critical factors involved in the analytical procedure, is a contribution toward the development of a standard asbestos analytical method.

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ABSTRACT

The analytical procedures used by a number of laboratories for the analysis of asbestos fibers in water samples have been investigated, with the overall objective of recommending an optimum technique.

Standardized aqueous dispersions of chrysotile, crocidolite and taconite tailings were prepared, samples of which were filtered so as to generate approximately 150 identically loaded membrane filters for each type of fiber. Filtration problems when using polycarbonate filters (Nuclepore) were solved, and an optimum filtration technique was developed which permits microscopically uniform deposits to be obtained. Such uniform deposits are essential, since only a small area of the filter is eventually examined and assumed to be representative. A large number of replicate analyses were made using the filters, to establish fiber losses and reproducibility of the various techniques. The preparation techniques investigated were:

(a) Carbon-coated Nuclepore with Jaffe washer dissolution;

(b) Uncoated Nuclepore with Jaffe washer dissolution;

(c) Both 0.22 μm and 0.45 μm pore size Millipore, with Jaffe washer dissolution;

(d) 0.45 μm pore size Millipore with condensation washer dissolution;

(e) Ontario Research Foundation (ORF) ashing technique. In this method a Millipore filter is first ashed and the ash redispersed in water. The resulting dispersion is filtered through a 0.1 μm Nuclepore which is then processed by technique (a).

The carbon-coated Nuclepore technique was found to have essentially zero fiber losses. Electron micrographs obtained both before and after the Jaffe washing operation show that all particles visible on the filter surface prior to washing can be located on the final replica. The Ontario Research Foundation ashing technique was also found to have essentially zero fiber losses for chrysotile and taconite, but a statistically significant 41% mean loss was found for crocidolite. The uncoated Nuclepore technique was found to give mean losses of up to 33%. There was some evidence that in the case of taconite, both the ORF ashing method and the Nuclepore-Jaffe method yielded final preparations in which the fibers were less aggregated.

In contrast, all of the Millipore techniques, using either the Jaffe washer or the condensation washer, produced mean fiber losses of up to 80%. For the 0.45 μm pore size Millipore preparations, no significant differences were found in fiber losses between the use of the Jaffe washer and the condensation washer. The results from the replicate Millipore samples indicate that these techniques are not reproducible in that coefficients of variation for 10 measurements as high as 0.8 were observed. Both of the Nuclepore techniques and the ORF ashing technique consistently achieved coefficients of variation better than 0.4.

The uniformity of the deposits on all the final electron microscope preparations was expressed quantitatively, and the most satisfactory performance was given by all of the Nuclepore techniques, both with and without the ashing step. The uniformity of the deposits using the Millipore techniques was generally very poor, indicating significant particle migration during the washing operations.

The effects of ultrasonic treatment at 20 kHz on fiber dispersions of both chrysotile and crocidolite were also evaluated. The fiber concentration of suspensions of chrysotile was shown to increase by about 0.2% per second at an ultrasonic power density of 0.5 watt/ml, and by about 0.1% per second at 0.05 watt/ml. No effect could be detected on crocidolite, even after 300 seconds.

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SECTION 1

INTRODUCTION

Asbestos is a term used to describe a variety of hydrated silicate minerals which have one common attribute: the ability to be separated into soft, silky fibers of colloidal dimensions. There is no single mineral known as asbestos; rather there are two major groups, containing a total of six varieties of minerals (1). The two groups are classified on the basis of their crystal structures: serpentine and amphiboles. The sole member of the serpentine class is chrysotile, which accounts for some 90% of the asbestos fiber produced today. There are five recognized types of amphibole asbestos: crocidolite, amosite, anthophyllite, tremolite and actinolite.

Chemically, chrysotile comprises hydrated magnesium silicate, $Mg_3.Si_2O_5.(OH)_4$ with minor inclusions of iron and aluminum oxides. The amphibole asbestoses are much more complex, being based on the general composition $(X)_7.Si_8\ O_{22}.(OH)_2$, in which X may be Na, Fe^{2+}, Fe^{3+}, Mg or Ca in various combinations. It is a common misconception that the asbestos minerals are "indestructible". In reality all are susceptible to both chemical and thermal degradation. Chrysotile is susceptible to acid attack, the degree and rate of dissolution being dependent on the temperature, concentration and nature of the acid, and also to some extent on the source of the chrysotile (2-3). In contrast to chrysotile, the amphiboles are more acid resistant, although there are significant differences between varieties. The resistance of asbestos minerals to attack by reagents other than acids is generally considered excellent at temperatures up to 100°C, but deteriorates rapidly at higher temperatures. Despite their relatively high fusion temperatures, asbestos minerals are all completely decomposed at temperatures of 1000°C or lower, depending on the variety. Independently of the surrounding atmosphere, chrysotile decomposes to form forsterite and silica at temperatures in excess of 500°C. However, finely sub-divided samples have been shown to dehydroxylate at much lower temperatures (4). The decomposition of amphiboles is extremely complex, and depends greatly on the surrounding atmosphere. Pyroxenes, cristobalite and iron oxide are frequent products of amphibole pyrolysis. Both chemical and thermal decomposition of asbestoses, even when complete, may leave decomposition products which retain the fibrous morphology of the original sample. This may lead to complications when an analysis relying on morphological characteristics alone is used without precaution.

The widespread use of asbestos containing materials gives rise to concern about exposure of the general population to low level concentrations of these materials in air, water supplies and food. Although hazards associated with the inhalation of asbestos at high concentrations are well established, (5,6,7) the significance of asbestos particles when ingested is not fully understood (8). However, there is some evidence of increased incidence of tumors of the gastro-intestinal tract where individuals have been exposed to the material over a long period (5,9). Methods are therefore required by which trace concentrations of asbestos minerals in water can be monitored.

Asbestos contamination can be reported in terms of either mass, or number of fibers, per unit volume of liquid. If the data are reported as mass concentrations, using for example, X-ray diffraction or infrared spectroscopy, no information is available about the dimensions of individual particles. On the other hand, microscopy techniques, which report number concentrations and number distributions, give detailed information about the size and shape characteristics of individual fibers. Epidemiological studies indicate that there appears to be a significant relationship between the degree of penetration of the fibers into tissue and the physical dimensions or aerodynamic diameters of the fibers (6). Similar size dependent phenomena have been suggested for ingestion of asbestos fibers (6,10). It is for this reason that fiber number concentrations, together with size measurements, should be determined, rather than simple measurements of the mass of asbestos fibers. The requirement that the number concentration be reported limits the analytical techniques available to either light or electron microscopy. Optical microscopy has for some years been used for industrial hygiene measurements of airborne particles in asbestos plants and mining operations. In principle, a membrane filter on which particles have been collected is examined by phase contrast microscopy at a magnification of approximately 450X, and all fibers longer than 5 micrometers are counted, regardless of their composition. Whilst this method is adequate as a guide for routine monitoring of air in asbestos processing plants, the environmental water sample presents an entirely different problem. Firstly, in most water samples the fibers observed are extremely small, and 5 micrometers would be a commonly encountered upper limit of length. Secondly, water usually contains a large amount of diatomaceous material, fragments of which can be mistaken for asbestos fibers. Thus for the environmental water sample, methods of identification of asbestos fibers are required.

The transmission electron microscope (TEM) has a resolution limit of the order of 0.2 - 0.5 nm, which is more than adequate for the observation of even the smallest fibers. The technique of selected area electron diffraction (SAED) is also available on this instrument, permitting investigations of the crystal structure of an individual fiber. More recently, it has become possible to incorporate the energy dispersive X-ray spectrometer (EDS) in the instrument, thus permitting information to be obtained about the chemical composition of the individual fiber. The use of the scanning electron microscope (SEM) for this purpose has also been suggested. Like the TEM, this instrument can also be fitted with an energy dispersive X-ray spectrometer. A typical resolution for a modern instrument would be about 10 nm. However, this resolution limit generally applies to an ideal, electrically conducting specimen. For the examination of asbestos fibers, non-conducting particles, with diameters down to 20 nm, are collected on a polymer filter surface. The filter is then vacuum coated with carbon or gold to prevent charging of the particles, and the overall contrast between the smallest fibers and the background is poor.

Additionally, the thickness of the gold or carbon coating can be of the order of 10 - 20 nm, expanding the apparent size of the fibers. Under these conditions the instrumental resolution limit is rarely achieved and the morphology of the narrowest fibers is difficult to observe. These sample-related resolution problems are largely associated with the analysis of chrysotile, rather than the amphibole fiber, since amphibole fibers are usually present in much larger sizes. A discussion of the applicability of the two instruments has been given by Ruud (11), who concluded that fewer errors are made in the identification procedure using the TEM-SAED combination than are made using the SEM-EDS combination.

Assuming that the TEM-SAED technique is to be used, the analytical problem is reduced to that of filtration of the water sample and transfer of a representative fraction of the retained solid material to a carbon-coated electron microscope grid. Various techniques by which this can be achieved have been reported. The techniques can be classified according to whether it is a direct preparation, or a preparation which incorporates an intermediate ashing step to remove organic materials. A direct method using an ultracentrifuge was reported by Durham and Pang (12), in which an ultra-centrifuge is used at an acceleration of about 30,000g to concentrate the solids from a one liter sample of water. The solids are then redispersed in a small volume of distilled water and an aliquot of this is transferred to a carbon-coated electron microscope grid using an microliter pipette. This technique has few advantages over filtration and has the disadvantage that the equipment is expensive. Extensive cleaning of the centrifuge containers is also required to ensure that there is no cross-contamination between samples. In a technique by Kramer and Mudroch (13), the water is first filtered through a Millipore or Nuclepore membrane filter, which is then given ultrasonic treatment whilst immersed in a small volume of fiber-free water. The filter is then removed and a drop of the remaining suspension applied using a microliter pipette to a carbon or collodion coated electron microscope grid. Using this technique, the efficiency of removal of fibers from the filter by the ultrasonic treatment can be questioned and the uniformity of the deposit on the final electron microscope preparation is also in doubt. A number of techniques are based on the work of Jaffe (14) and Kalmus (15), in which the particulate material on the surface of a membrane filter is directly transferred to a carbon-coated electron microscope grid by solvent dissolution of the membrane filter. The solvent dissolution can be achieved using either a Jaffe washer or a condensation washer. In a technique reported by Cook (16), a Nuclepore filter is used for the original water filtration, after which it is carbon-coated by vacuum evaporation. The filter is then dissolved by the Jaffe washer technique, leaving a carbon extraction replica of the original filter surface, in which the particulate material is embedded. Techniques incorporating an ashing step to remove organic materials have been reported by Biles and Emerson (17), Cunningham and Pontefract (18), Chatfield (19) and Chatfield and Glass (20). The requirement for the ashing step arises when there is significant content of organic material in the water. Attempts to produce samples by any of the direct filtration techniques may result in a large deposit of slime on the surface of the filter which contains the inorganic particles. Alternatively, the requirement for all particles to be separate and in a single layer on the final microscope preparation may involve a dilution such that the detection level for asbestos in the presence of this other material is totally unacceptable. In these cases, oxidation of the organic content has to be achieved prior to the preparation of the electron microscope sample. Biles and Emerson (17) and Cunningham and Pontefract (18) used a muffle furnace, in which a filter or a centrifugate was oxidized at high temperatures. More recently, low temperature plasma ashing has been applied to this problem. The technique of Chatfield and Glass involves initial filtration of the water sample through a 0.1 µm Millipore membrane filter, which is then ashed in a low temperature asher. The ash is redispersed ultrasonically in a small volume of double distilled water, and this suspension is filtered through a 0.1 µm pore size Nuclepore filter. This filter is then prepared for the electron microscope by the carbon-coating replication process.

The consensus of opinions in the U.S.A. and the majority in Canada now favor the filtration methods and direct transfer techniques for preparation of electron microscope grids. In this report the results of an investigation of the quantitative performance of these methods are presented. For those samples containing large amounts of organic materials an alternate technique incorporating an ashing step is required. The most promising technique from those available appears to be that of Chatfield and Glass; this method was investigated along with the direct transfer techniques. All of the analytical methods referred to occasionally require the use of ultrasonic treatment. In some cases, if a water sample has been allowed to stand for any length of time, the particulate material, including the asbestos fibers if present, may have coagulated and possibly also been deposited on the interior surface of the container. In such cases ultrasonic treatment must be used to ensure representative sampling from the container. In addition, all techniques which incorporate an ashing step require the use of ultrasonic treatment in order to redisperse the ash. There is little published work on the effect of ultrasonic treatment on dispersions of asbestos fibers, but work by Chatfield and Glass (21) indicates that at low power densities the effects are minimal on dispersions of chrysotile asbestos. Since ultrasonic treatment is an essential step in some preparation techniques, the effects of such treatment on the fiber number concentrations and their size distributions are investigated in a quantitative manner.

SECTION 2

CONCLUSIONS

The carbon-coated Nuclepore technique has essentially zero fiber losses, and particles remain fixed in position during the Jaffe washing operation. This technique gives a narrower spectrum of results for replicate analyses than any of the Millipore direct dissolution methods.

The ORF ashing technique yields a spectrum of results and grid uniformity similar to those of the carbon coated Nuclepore method. Any losses which occur do so in the ashing step, and the subsequent preparation procedure has essentially zero losses. Fiber losses were negligible for chrysotile and taconite, but a mean loss of 41% occurred in the crocidolite preparations. The reason for the crocidolite loss is not clear, but may be associated with the surface properties of such small amphibole fibers. There is some evidence in the crocidolite fiber length data to suggest a preferential loss of the smaller fibers. In the case of the taconite dispersion, the fibers were generally somewhat longer, and any such effects were not noticed. Both of the Nuclepore techniques and the ashing technique are superior to the Millipore preparations in that they have generally lower or even zero fiber losses, and give a significantly more uniform fiber deposition on the final electron microscope grids.

All three Millipore dissolution techniques introduce large preparation losses, regardless of whether the Jaffe washer or the condensation washer is used. Significant particle movement occurs during dissolution of the filter: this particle movement leads to non-uniform deposition of fibers on the final electron microscope grids. The losses when using the Jaffe washer were not significantly different from those using the condensation washer for the 0.45 µm pore size Millipore filter.

There is less fiber loss when using the 0.22 µm pore size Millipore filter than when the 0.45 µm filters are used in the Jaffe washer dissolution process. This is possibly due to a shallower penetration of the particulate into the 0.22 µm filter structure, and thus more efficient deposition of the material on the carbon-coated grids. This conclusion is supported by the observation of chrysotile contamination in the Millipore filters when analyzed by the ashing technique, while such contamination was not detected by the direct transfer Millipore techniques.

The fiber loss using the uncoated Nuclepore preparation technique was significantly less than those when using the direct transfer Millipore techniques.

At the 5% significance level, an increase in the fiber concentration was detectable when a chrysotile asbestos dispersion was exposed to ultrasonic treatment. If an approximately linear effect with time is assumed, at 0.05 watt/ml the numerical increase in fiber concentration was about 0.1% per second. At 0.5 watt/ml the corresponding increase was about 0.2% per second. Even after treatment at 0.5 watt/ml, undispersed bundles of chrysotile were still evident.

At the 5% significance level, no numerical increase in fiber concentration was detected in the case of crocidolite. In neither case could a systematic change in size distribution be detected.

SECTION 3

REVIEW OF TECHNIQUES INVESTIGATED

3.1 CARBON COATED NUCLEPORE TECHNIQUE

The Nuclepore filter consists of a polycarbonate material which is soluble in chloroform. This type of filter is unique, in that it consists of a continuous, featureless plastic film, perforated by cylindrical holes of a narrowly defined size range. Figure 1 shows a scanning electron micrograph of a 0.1 μm pore size Nuclepore filter. It can be seen that the surface structure of this filter would present no obstacles in the identification of particles on its surface.

Using this technique, a small volume of the water in question is first filtered, after which the filter is dried and carbon coated using a vacuum evaporator. A small square of the coated filter is placed on a 200 mesh copper electron microscope grid, and the filter dissolved away using chloroform. For the dissolution process, two designs of Jaffe washer are available. Figure 2 shows the design of washer used by Kalmus (15), which has proved satisfactory in many fields of application since 1954. It consists of a supporting bridge, made from a rectangular strip of stainless steel wire mesh bent sharply to form an inverted "U". The upper flat surface is covered lengthwise with a paper strip cut from a Whatman filter of slightly smaller width than that of the bridge. The end of the Whatman paper strip is bent downwards so as to touch the floor of the petri dish in which it is placed. Grids are placed in the position illustrated, on the top of which are placed portions of the carbon-coated Nuclepore filter. The lid of the petri dish is then placed in position and the assembly allowed to stand for periods of up to two days, after which the plastic filter medium is completely dissolved, leaving a thin carbon film containing the embedded particulate. The alternative design is shown in Figure 3, in which a pile of 4 or 5 glass microscope slides is placed in the bottom of a petri dish. These are covered by two or three Whatman filters in the position shown, which in turn are covered by a flat strip of stainless steel mesh. The grids are placed on top of the stainless steel mesh and the assembly is used generally in the same manner as the other design.

3.2 DIRECT DISSOLUTION OF NUCLEPORE FILTERS ONTO CARBON-COATED GRIDS

In this technique, particulate from the surface of an uncoated Nuclepore filter is deposited by direct transfer to a vacuum evaporated thin carbon film, which is in turn supported by a 200 mesh copper electron microscope grid. A number of techniques are available for preparation of the carbon-coated grids

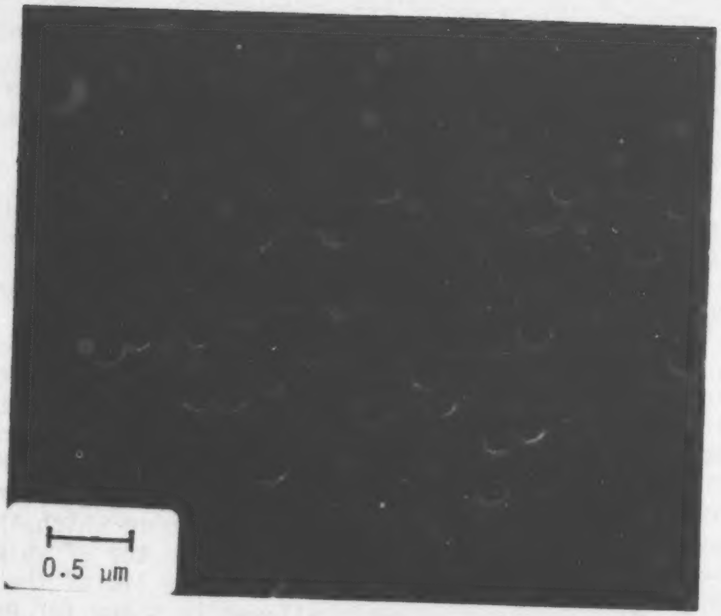


Fig. 1 Scanning Electron Micrograph of 0.1 μm Pore Size Nuclepore Filter Surface (Cambridge Stereoscan S4, 20 kV, gold coated)

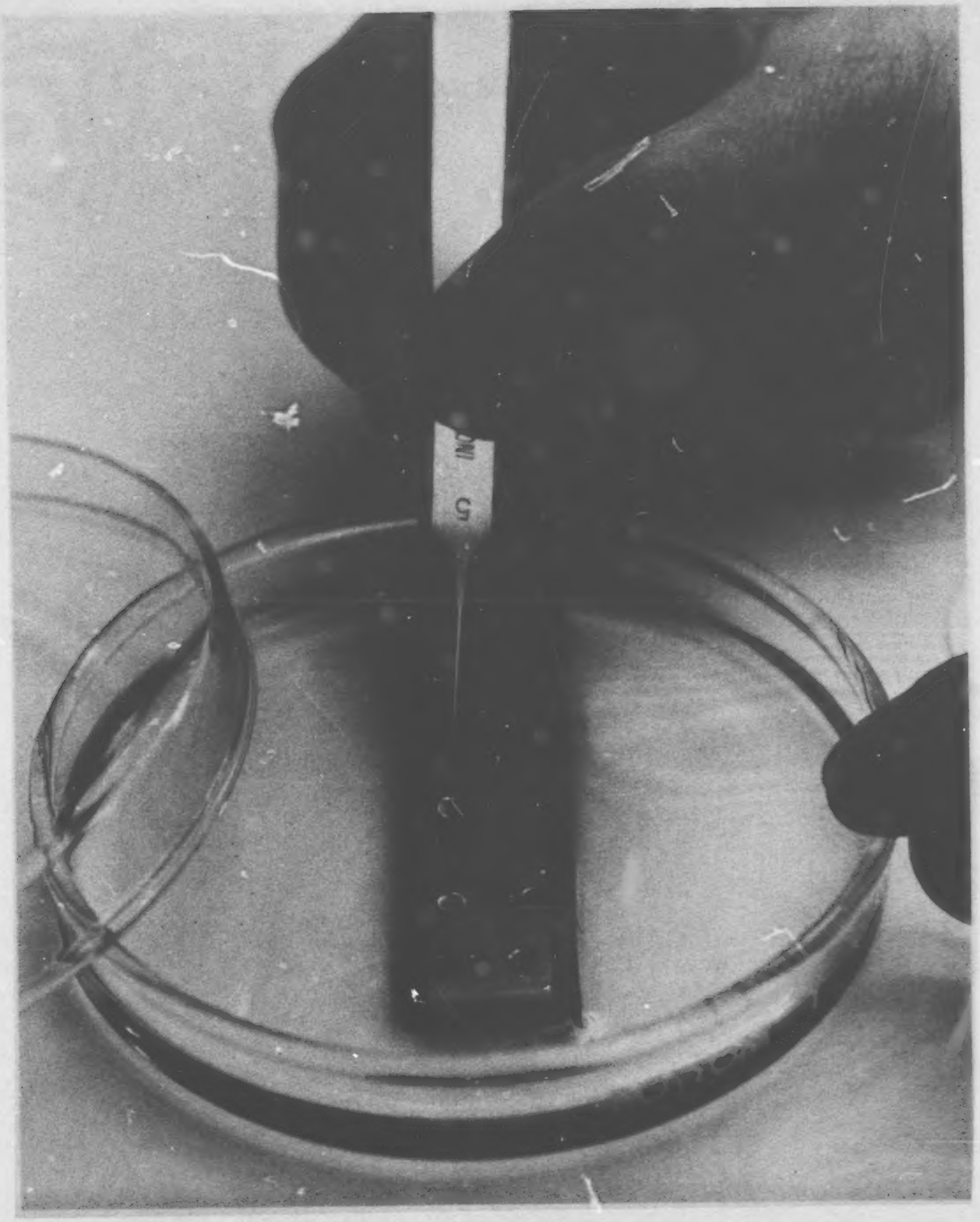


Fig. 2 Jaffe Washer

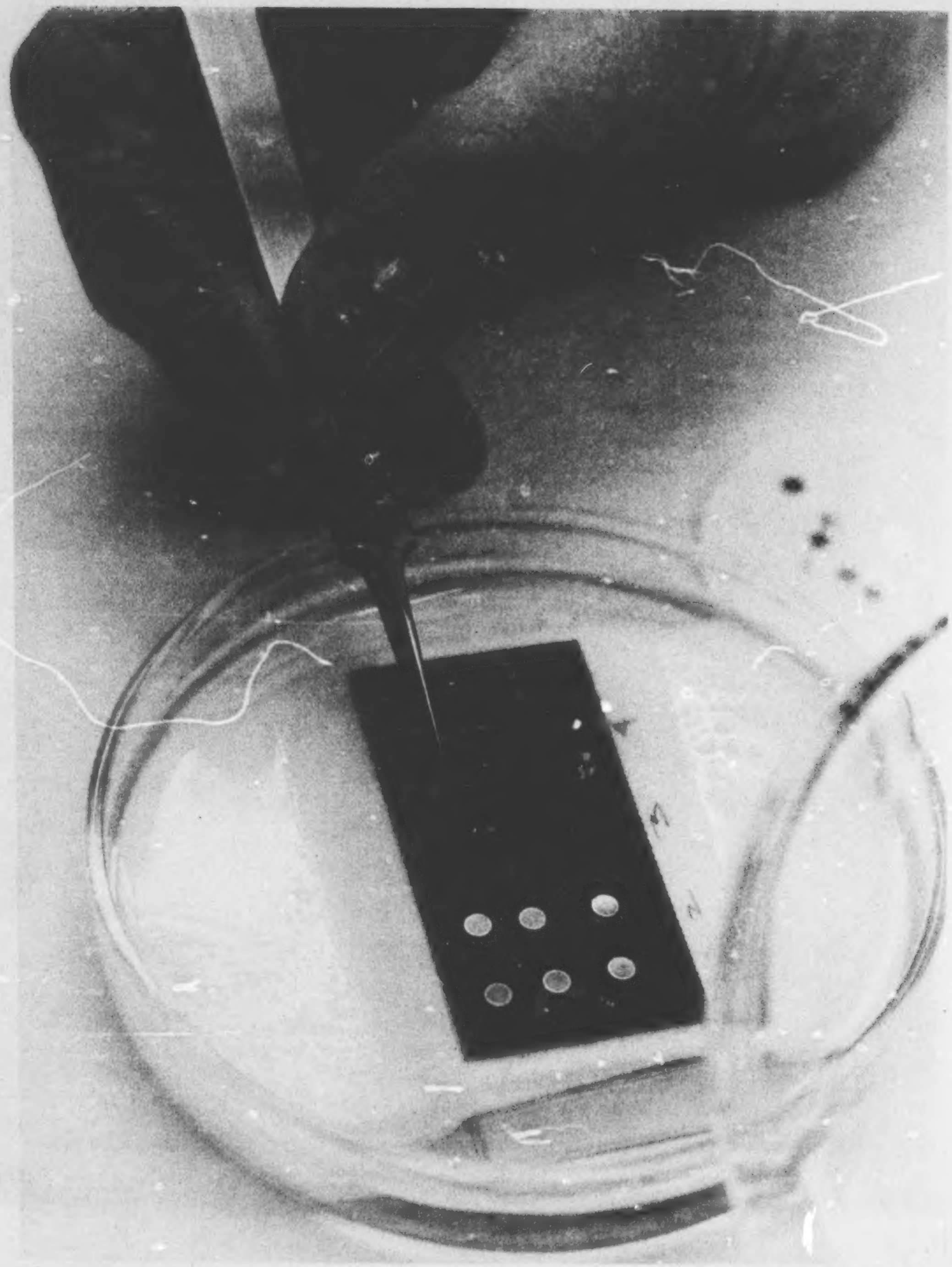


Fig. 3 Alternative Design of Jaffe Washer

required for this technique. A suitable and reliable method is to cast a single drop of a 1 or 2% solution of parlodion in n-pentyl acetate onto a clean water surface contained in a crystallizing dish. After the solvent has evaporated, a thin film of nitrocellulose is left floating on the water surface. A number of copper electron microscope grids are placed on the surface of this floating plastic layer, and a Whatman filter paper is then dropped onto the water surface. The Whatman filter paper may then be lifted from the surface of the water, when it brings with it the plastic film and the grids. This assembly is allowed to dry and carbon coated in the vacuum evaporator. The copper grids, supporting a carbon coated nitrocellulose film, can be picked from the surface of the paper. These grids are then placed in a Jaffe washer, using n-pentyl acetate as the solvent. The nitrocellulose layer is thus dissolved away from the grids, leaving only the carbon film. This technique produces nearly perfect carbon-coated grids, having very few areas in which the carbon film is broken. A large number of coated grids can be prepared at one time, and they do not appear to deteriorate in storage.

3.3 JAFFE WASHER DISSOLUTION OF 0.45 μm & 0.22 μm PORE SIZE MILLIPORE FILTERS

Figure 4 shows an SEM micrograph of a Millipore filter surface. In contrast to the Nuclepore filter, there is much surface detail, and if replicated by carbon evaporation, particles would be difficult to locate within it. Using this dissolution technique, a small square of the Millipore filter is placed, deposit side down, onto a carbon coated copper grid in a Jaffe washer, using acetone as the solvent. Beaman and File considered that fiber losses associated with Jaffe washer preparations were small (22) and in general less than 10%. However, they used tetrahydrofuran as the solvent, rather than acetone which is more commonly used with this technique.

3.4 CONDENSATION WASHER TECHNIQUE

This technique has been used by Beaman and File (22), McCrone and Stewart (23) and McFarren and Millette (24). Figure 5 shows a condensation washer. Essentially it consists of a heated flask containing solvent, vapor from which is prevented from escaping by a vertical condenser. A side arm permits entry of a water-cooled cold finger, on which the membrane filter and carbon-coated grid assemblies are placed. Dissolution of the filter takes place over a period of some hours by a reflux washing action. The original purpose of this device was for the dissolution of plastic from carbon-coated plastic replicas where loose particle movement is of no consequence. It has since been applied to the preparation of particulate samples with varying degrees of success. Opinions as to the proper operating conditions for the condensation washer vary, but there is agreement that fiber losses will at least be minimised if the washer is operated with the condensation level approximately at the position of the grids. Rapid operation will result in violent condensation action in the vicinity of the grids, and will inevitably result in fiber losses. A brass holder device, designed by Millette, permits easier handling of the carbon-coated grids and filter portions when assembling the washer. It has also been suggested that the condensation washer with chloroform as the solvent could be used to dissolve carbon coated Nuclepore filters. The

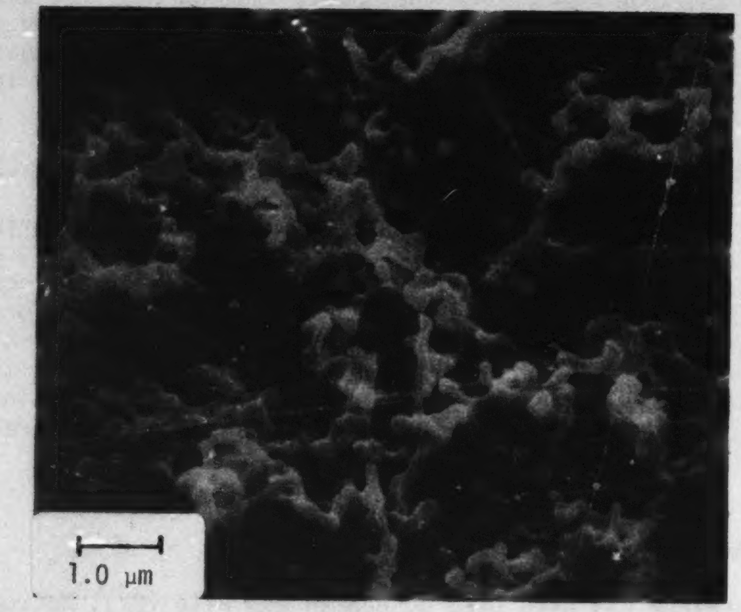


Fig. 4 Scanning Electron Micrograph of Millipore Filter Surface (Cambridge Stereoscan S4, 20 kV, gold coated)

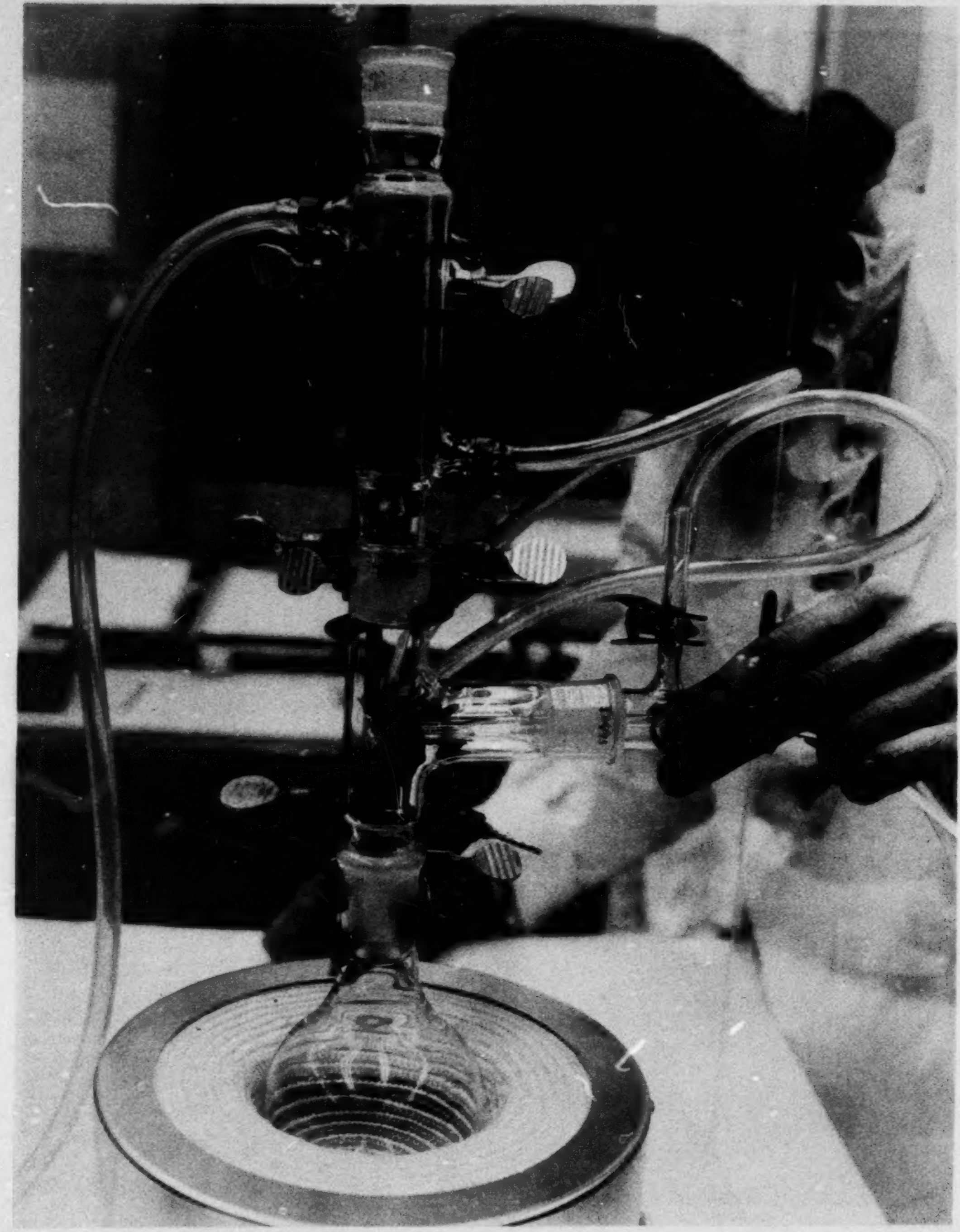


Fig. 5 Condensation Washer, Shown Inside Protective Draft Shield

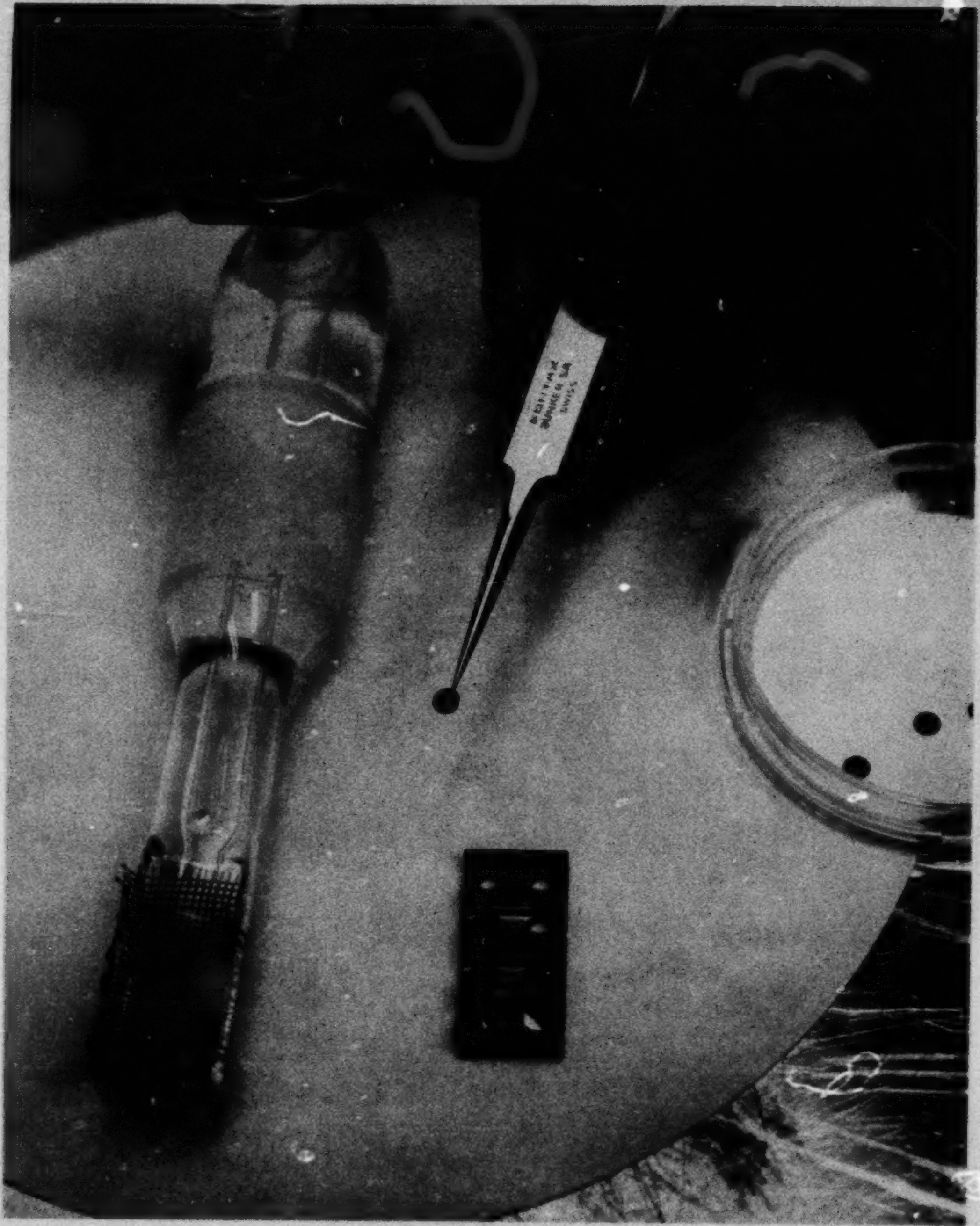


Fig. 6 Detail of Condensation Washer Cold Finger and Brass Specimen Grid Holder

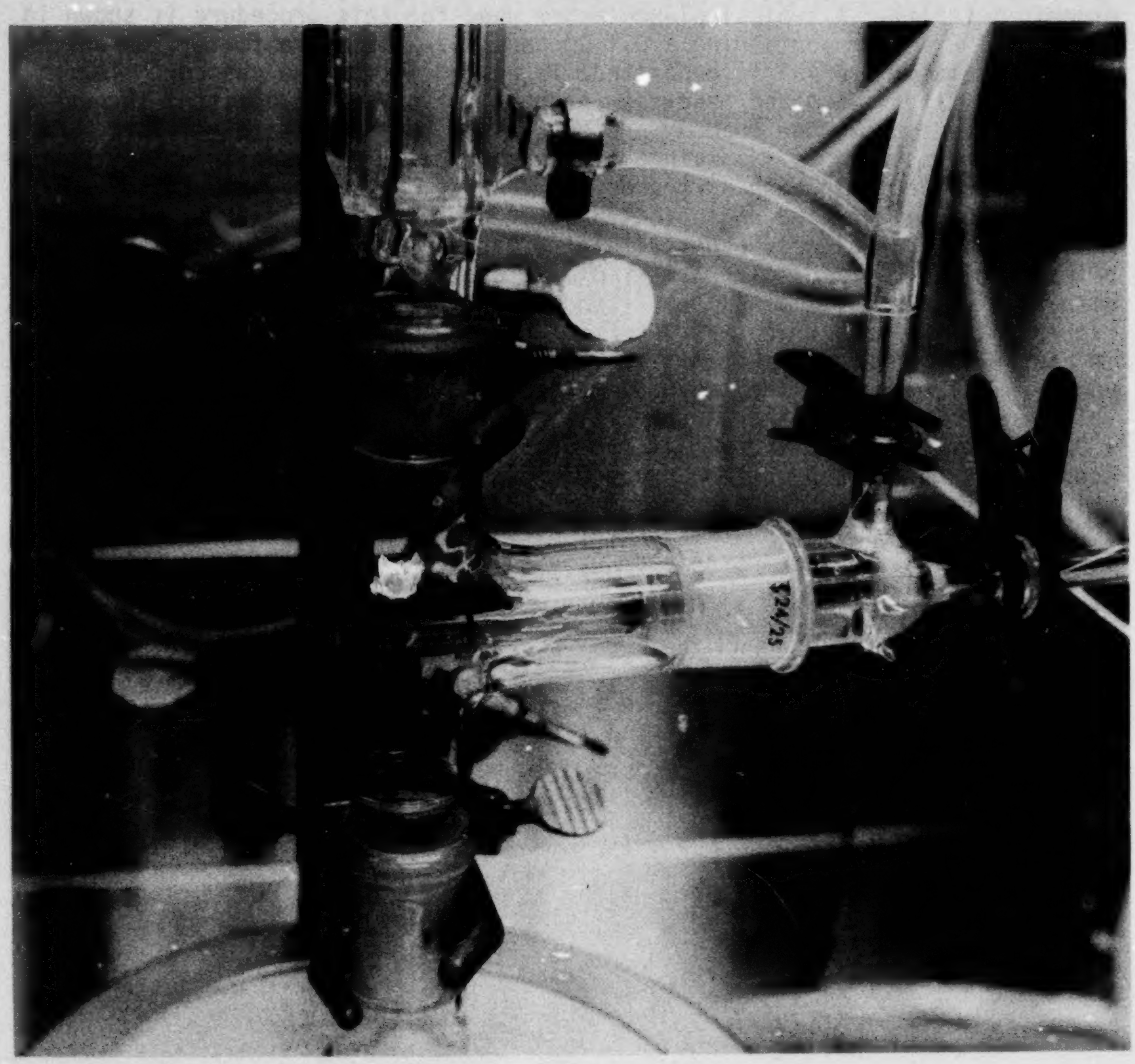


Fig. 7 Condensation Washer Cold Finger in Position

feasibility of the latter approach was investigated in this program.

3.5 ONTARIO RESEARCH FOUNDATION ASHING TECHNIQUE

This technique was reported by Chatfield and Glass (20,21) as an optimum technique for the analysis of water supplies having high concentrations of organic material. A typical plasma asher used for this procedure is shown in Figure 8. A beaker containing a 0.1 μm pore size Millipore filter, on which the particulate material is deposited, is placed inside the chamber. The chamber is first evacuated, and then a supply of oxygen is admitted through a needle valve so that the chamber pressure rises to approximately 1 Torr. An electrical discharge is maintained by the absorption of radio frequency energy supplied from a coil which surrounds the chamber. At an oxygen pressure of approximately 1 Torr and an indicated power of approximately 20 W, Millipore filters can be completely ashed within 24 hours. Figure 9 shows beakers being removed from a single chamber plasma asher. To obtain adequate redispersal of the ash, it has been found necessary to use an ultrasonic probe, rather than the bath which was reported previously. A suitable probe is shown in Figure 10; this unit is capable of generating 40 or 50 W of ultrasonic energy. After ashing, the residual material is redispersed in a small volume of double-distilled water. This suspension is then filtered through a 0.1 μm pore size Nuclepore filter, which is prepared for the electron microscope using the carbon-coating procedure discussed earlier. The validity of this preparation technique is, of course, contingent on a successful demonstration that the ultrasonic treatment used does not significantly change the fiber dimensions or concentrations reported.

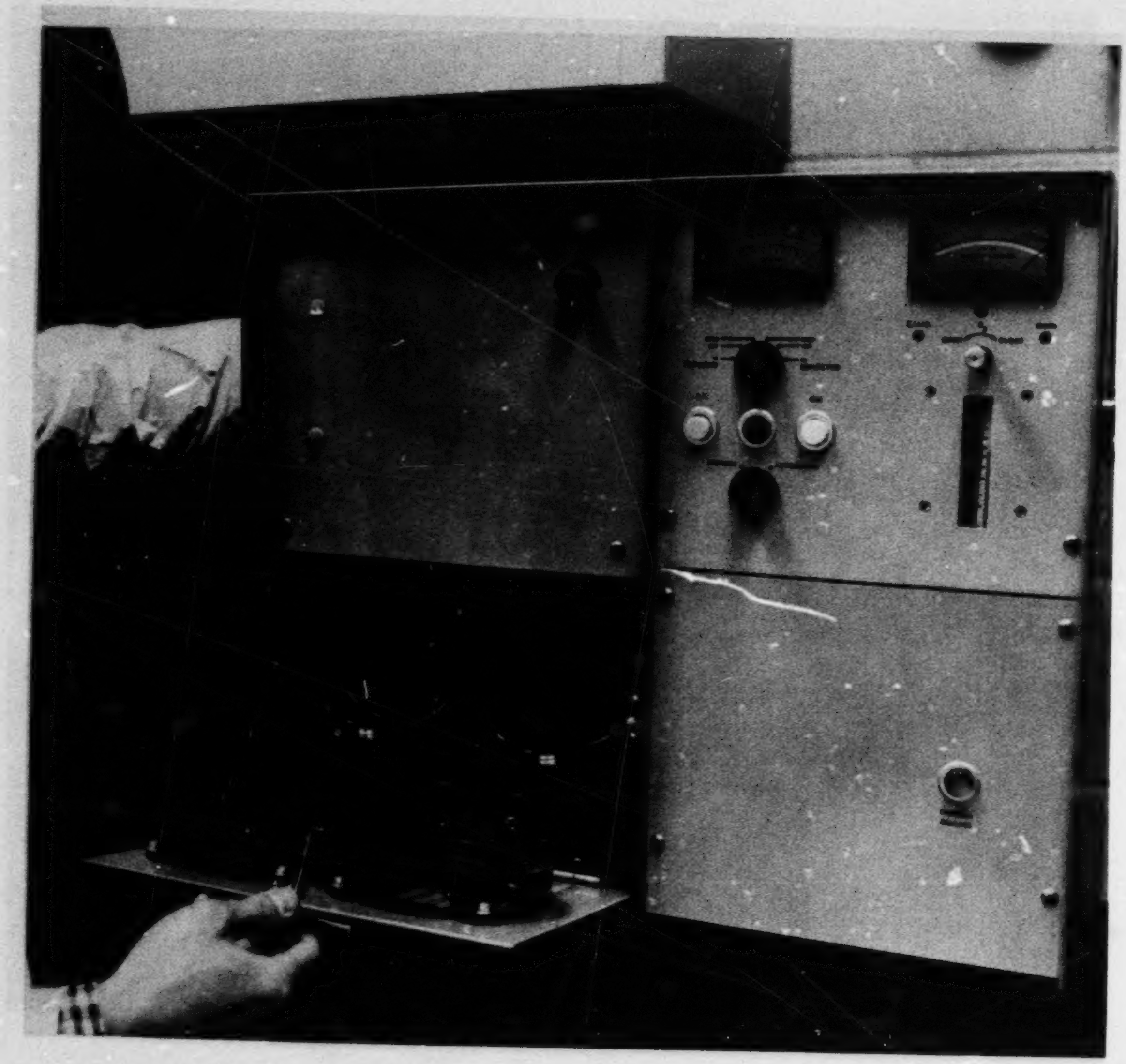


Fig. 8 Low Temperature Plasma Asher (Two Chamber Type)

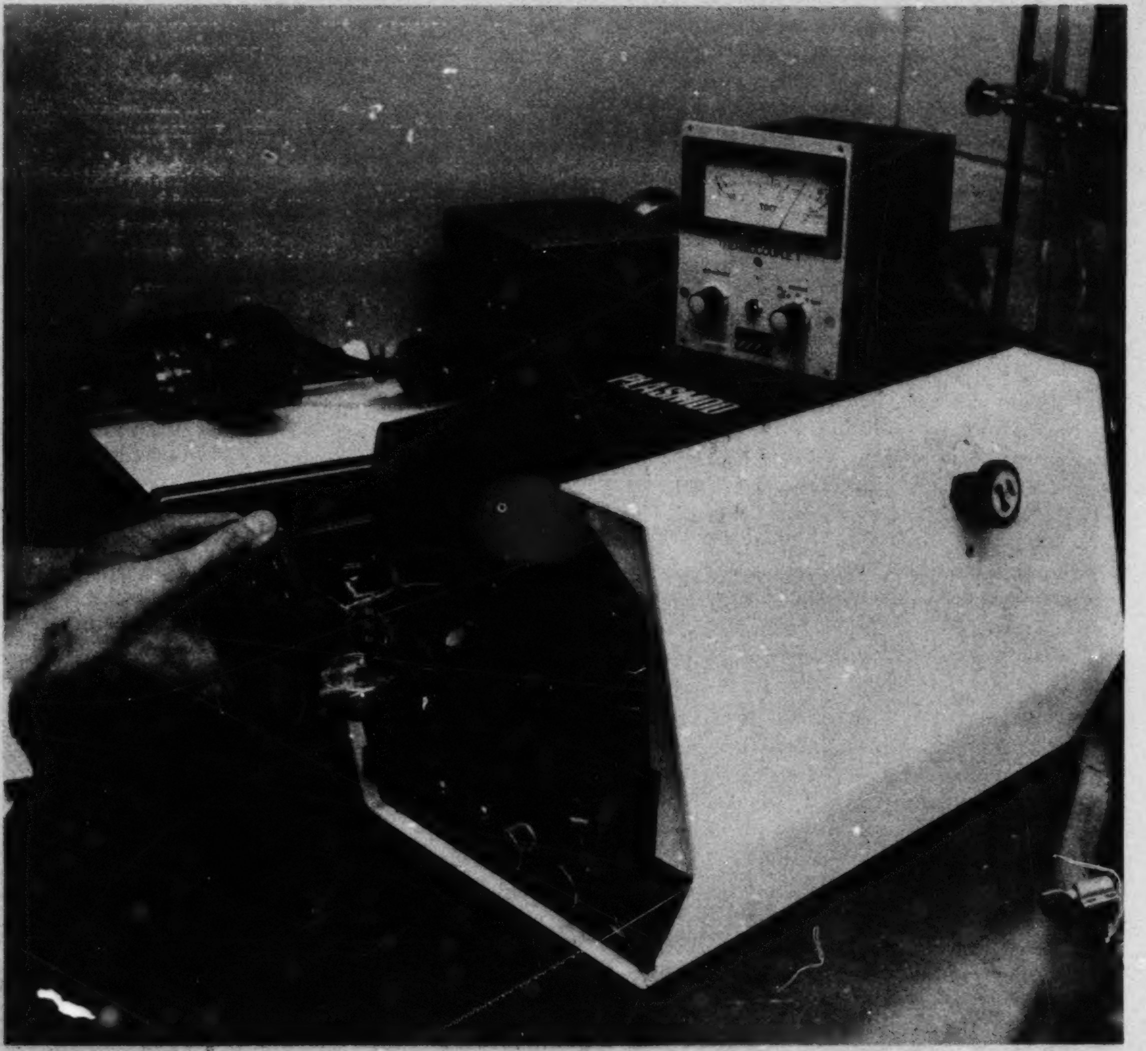


Fig. 9 Ashed Samples in Beakers Being Removed from Single Chamber Asher

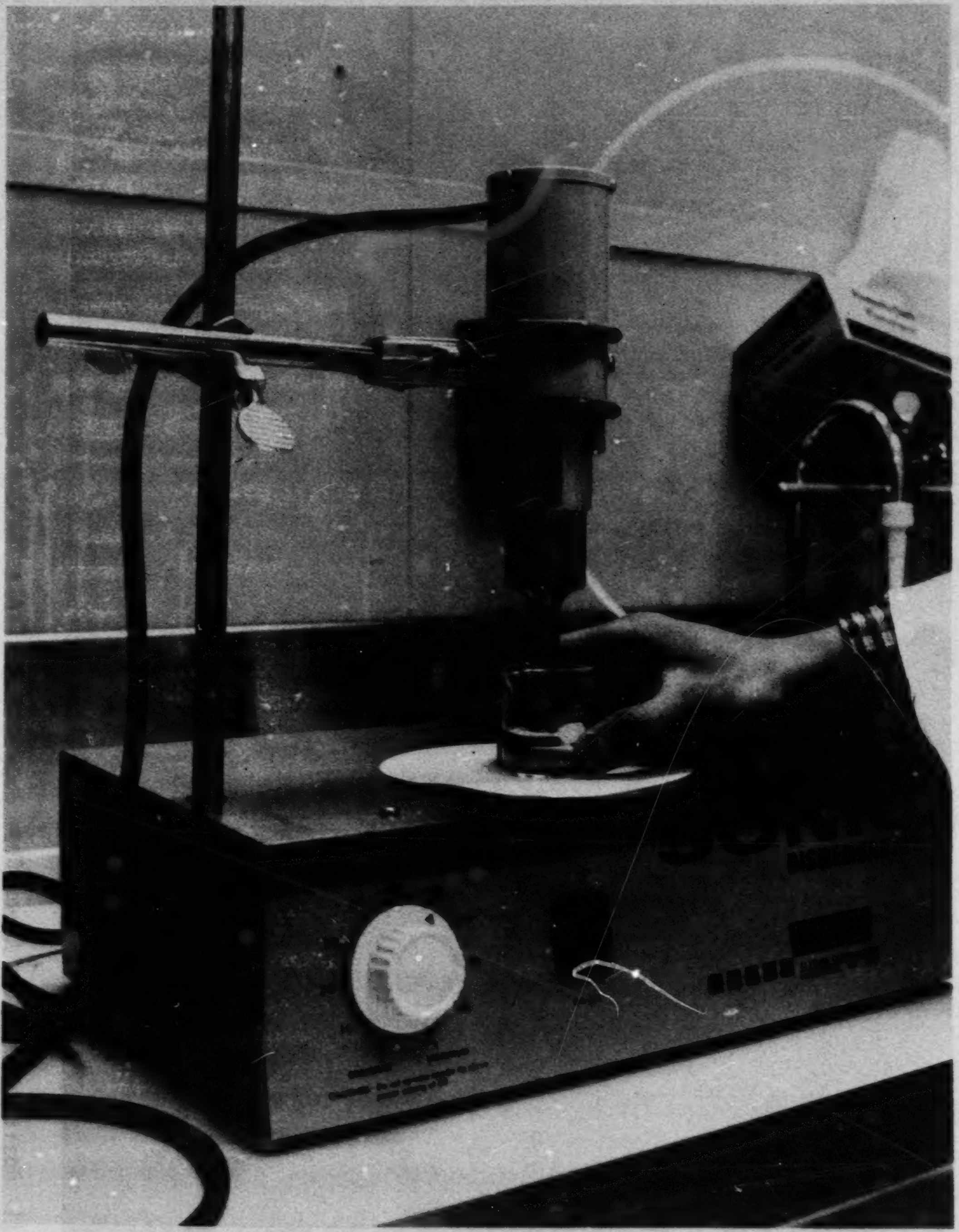


Fig. 10 Ultrasonic Probe for Redispersal of Ashed Residues

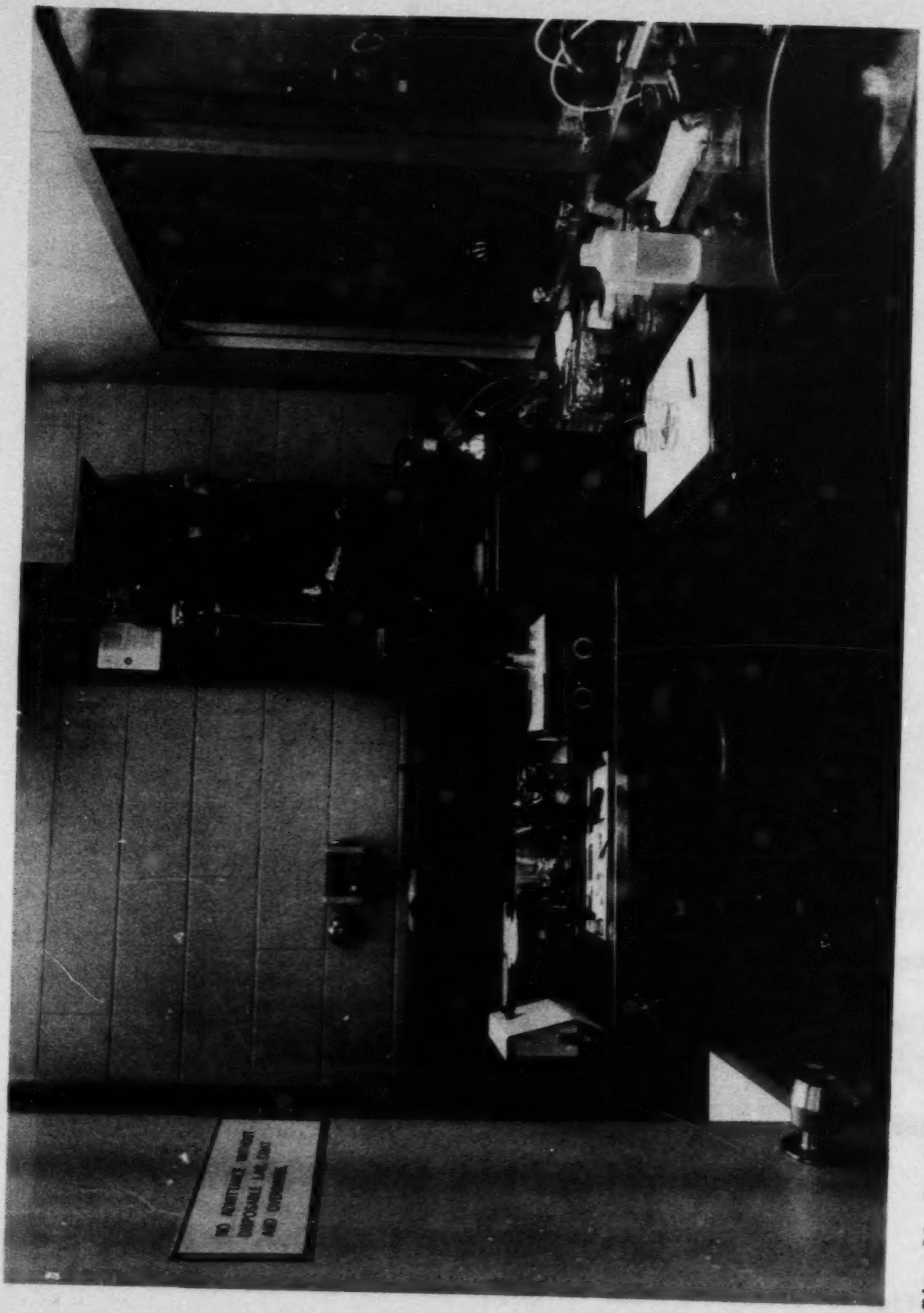


Fig. 11 Positive Pressure Clean Room Required for Contamination Control During Specimen Preparation

SECTION 4

EXPERIMENTAL APPROACH

The principal objectives are to establish the reproducibility and fiber losses when using the various methods of electron microscope sample preparation. In order to achieve this, a large number of membrane filters, both Millipore and Nuclepore, were loaded with identical concentrations of asbestos fibers. For the Millipore filter, 0.22 µm and 0.45 µm pore sizes were selected, whilst for the Nuclepore filter the 0.1 µm pore size was used. Three types of asbestos fibers were investigated: UICC chrysotile, UICC crocidolite, and a suspension of taconite tailings obtained from the Duluth area of Minnesota. In order to eliminate as many experimental errors as possible, all of the filtrations were carried out by one person, using standardized techniques. Since the stability of the standard fiber suspensions was questionable, the filtrations for each type of asbestos fiber were all performed as rapidly as possible, shaking the stock suspension between samples. In fact, with proper organization, it was possible to prepare each series within a period of one day. All of the sample preparation was carried out by one person. So far as scheduling would permit, fiber counting was generally performed by the allocation of one material and one instrument to one person; for example, all of the taconite samples were counted by a single person using one instrument only. In this way, when attempting to compare different preparation techniques for the same type of fiber, differences between individuals in fiber counting philosophy were eliminated.

In order to control contamination, all sample preparation was performed in an asbestos-free clean room, maintained at positive pressure by air supplied through filters and electrostatic precipitators. Figure 11 shows the clean room, in which can be seen a low temperature plasma asher, a multiple filtering manifold, an ultrasonic probe and a glass water still which is used to redistill the primary supply of distilled water. All glassware and equipment used is washed using double distilled water. For the actual fiber counting, chrysotile fibers were classified on the basis of morphology alone, since this was the only material present. Crocidolite and taconite fibers were categorized as confirmed amphibole, probable amphibole, and the sum of these. Fibers placed into the "confirmed" category yielded selected area electron diffraction patterns recognizable and similar to those from reference amphibole fibers. Fibers giving either no pattern, or sufficient diffraction spots for reliable identification, were placed into the "probable" category. Initially, approximately 10 samples were processed for each type of asbestos fiber, and each preparation technique. On the basis of the results, an optimum technique was selected; an additional 10 filters for each fiber type were then analyzed using this technique, in order to establish better statistical validity. The technique which incorporates the ashing step required an investigation into the effects of ultrasonic treatment on the fibers. This investigation was carried out for both chrysotile and crocidolite dispersions. Effects of the ultrasonic treatment were anticipated to be most serious in the case of chrysotile, and for this part of the work a natural water sample was used, originating from a contaminated lake in Northern Ontario. This sample was ideal for the purpose, since it contained many fiber bundles. In the absence of any suitable natural specimen, the crocidolite sample was prepared from UICC material, avoiding the use of ultrasonics in this particular case.

SECTION 5

PREPARATION OF STANDARD DISPERSIONS

Experience has shown that it is almost impossible to estimate the final numerical concentration of a fiber dispersion prepared artifically from the solid material, particularly if only the shorter fibers are required. Furthermore, such dispersions are usually unstable and cannot be relied upon for more than a day or so. Previous work (20,21) indicated that natural lake water samples, spiked with artificial dispersions of chrysotile, crocidolite and taconite tailings, remain stable for periods of some months. However, later work in methodology investigation has shown that dispersions of these materials in distilled water are not particularly stable, and that flocculation occurs, giving rise to difficulty in obtaining a representative sample. In order to prepare a large number of identically loaded filters, therefore, it is necessary to prepare the dispersion and then to perform the filtrations in a very short time.

For preparation of the chrysotile and crocidolite dispersions, approximately 5 mg of the UICC fiber was placed into a clean agate pestle and mortar; the material was then hand ground in about 1 ml of double distilled water. This suspension was transferred to 1 liter of double-distilled water in a 1.5 liter beaker, and stirred gently with a glass rod to break up the aggregates. Caution was exercised in stirring, since grinding of a glass rod on the inside of a beaker can generate a surprising amount of glass powder. The dispersion was then exposed to ultrasonic treatment, using a probe, for approximately 5 minutes at a power density of 0.05 Watt/ml, after which the suspension was allowed to stand overnight. Using a large pipette, the top 500 to 700 ml was transferred to a 1 liter polyethylene bottle. The remainder, including the settled material, was discarded. This dispersion formed the concentrated stock suspension. A 10 ml sample of this stock was dispersed in 3 liters of double-distilled water, giving the final fiber dispersion. A small volume of this was filtered onto a Nuclepore filter and examined by the carbon-coating technique in order to establish the approximate fiber concentration. A calculation was then made to determine how much of the dispersion should be filtered so as to produce the required filter loading. The target concentration was that equivalent to approximately 100 fibers in ten grid openings of the final electron microscope sample. For chrysotile, it was found necessary to filter 20 ml and for crocidolite 15 ml. For the work on Cummingtonite, a suspension of taconite tailings was obtained from Dr. P.M. Cook of the National Water Quality Laboratory, Duluth, Minnesota. This stock suspension was diluted 1:10, and 6 ml of this was dispersed in 3 liters of double-distilled water. The required filter loading was obtained by the filtration of 10 ml of the resulting suspension. It is necessary to emphasize that synthetic fiber dispersions of chrysotile and crocidolite, and probably taconite, are usually quite unstable and that the only reliable method of obtaining a reproducible standard is by fixation of a known volume of such a suspension on filters. On the other hand the work of Chatfield and Glass (20,21) demonstrated the stability of some natural samples when spiked with artificial fiber dispersions. In effect, a fiber dispersion in water may be stable, but its stability appears to be a complex function of many variables and should not be relied upon.

SECTION 6

FILTRATION

Although no particular problem has been demonstrated when using the Millipore type of membrane filter, filtration is undoubtedly the most critical step in the Nuclepore preparation procedures. Filtration is performed using commercially available 1 inch diameter assemblies, consisting of filter funnels with a sintered glass frit support, and liquid reservoirs with vertical sides. Using vertical sided reservoirs, the geometry is optimized so as to minimize preferential deposition of particulate as a function of position on the filter. Figure 13 shows an optical micrograph of a Nuclepore filter which has been used to filter a concentrated dispersion of taconite tailings. It can be seen that the deposit is extremely non-uniform, with some areas having little or no deposit at all. It has been found experimentally that in the absence of any precautions this is the type of deposit which may be expected if a Nuclepore filter is used in one of these filtration assemblies. Figure 14 shows a scanning electron micrograph of such a taconite deposit on a Nuclepore filter. It can be seen that significant areas, comparable with that of a 3 mm diameter electron microscope grid, could be extracted from such a filter by direct transfer techniques so as to give a totally unrepresentative idea of the filter loading. A heavy deposit of this type permits the non-uniformities to be recognized. However, at the filter loadings normally used for electron microscope sample preparation, such irregularities would go unnoticed and could easily lead to some of the unsatisfactory inter-laboratory comparisons which have so far been conducted (25). This observation is extremely worrying, since the direct transfer techniques of specimen preparation rely on absolute uniformity of particulate material deposit over the active area of the filter. It was necessary to investigate this effect before the standard series of filters could be prepared. Chatfield and Glass (20) observed this type of non-uniform deposition when filtering redispersed ash on to Nuclepore filters. Assuming the effect to be a property of the suspension, a detergent was added and this technique appeared to yield a uniform deposit. Later ethyl alcohol was also used satisfactorily as the redispersal medium, together with an alcohol resistant Millipore as a backing filter for the Nuclepore. However, the addition of detergent was considered inadvisable when the Nuclepore was to be subsequently carbon-replicated, since there is an increased possibility of detachment of particles from the carbon replica. A technique for satisfactory filtration of water samples was therefore still required.

The principal origin of the non-uniformity can be envisioned by inspection of Figure 15, in which is shown a scanning electron micrograph of the sintered glass frit used to support the Nuclepore filter during the filtration process. Areas of filter closely contacting the flat ground areas

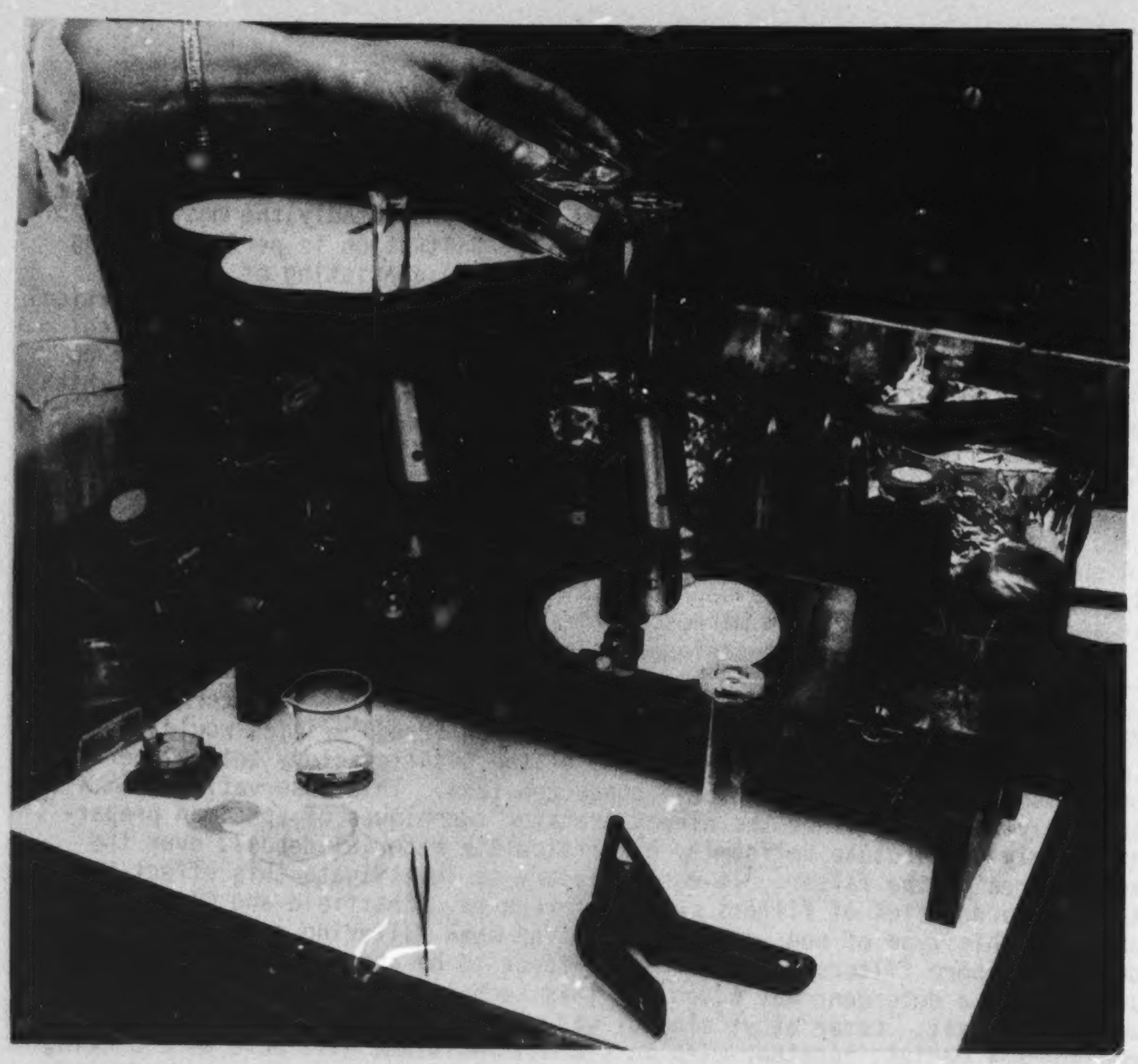


Fig. 12 Filtration Manifold in use for Simultaneous Filtration of Water Samples

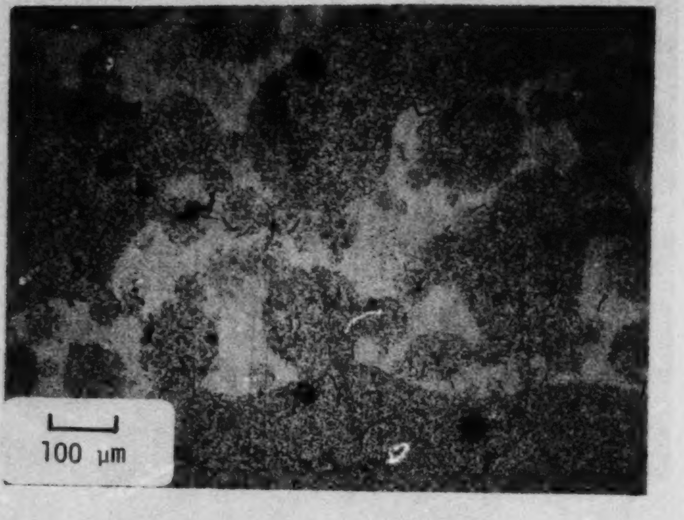


Fig. 13 Optical Micrograph of Nuclepore Filter Showing Uneven Deposit of Particulate

Fig. 14 SEM Micrograph of Taconite Deposit on Nuclepore Filter, Showing Uneven Deposit of Particulate (Cambridge Stereoscan S4, 20 kV, gold coated)

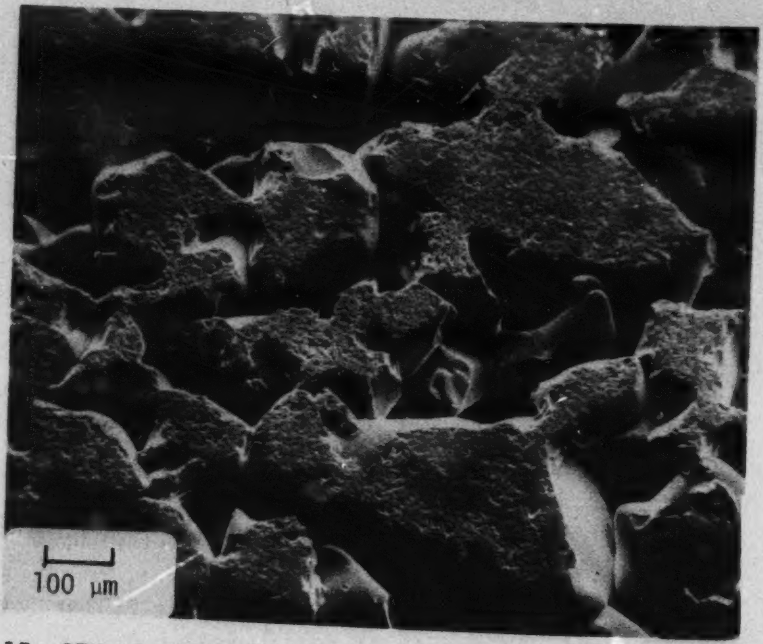


Fig. 15 SEM Micrograph of Glass Frit Filter Support, Showing Flat Areas Which Restrict Filtration Rate Locally (Cambridge Stereoscan S4, 20 kV, gold coated)

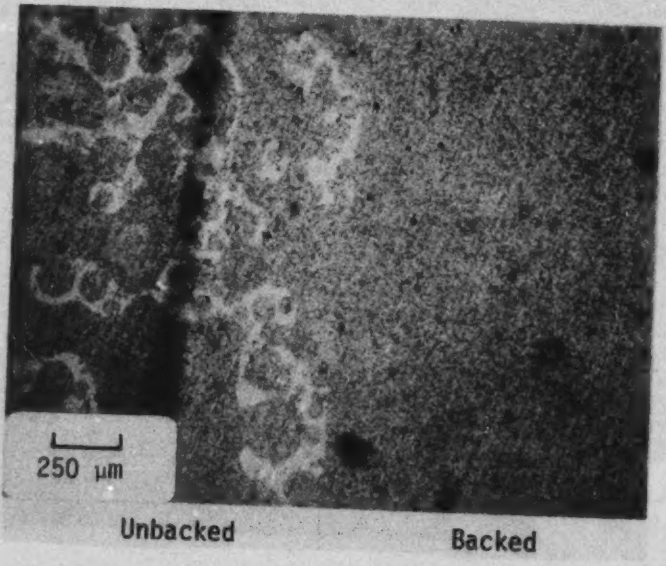
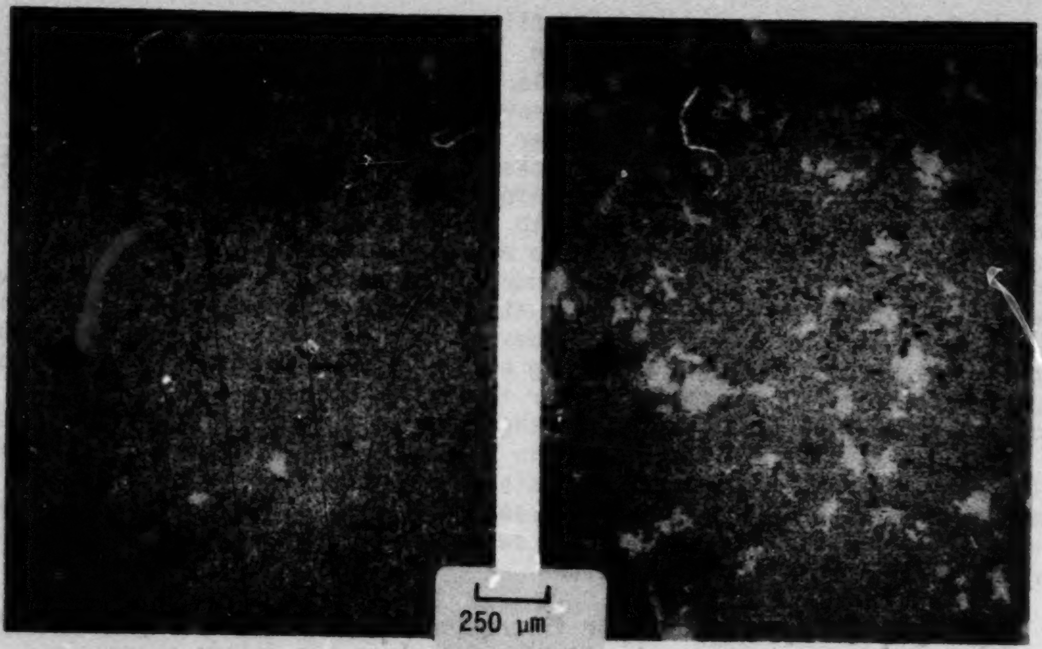


Fig. 16 Optical Micrograph of Nuclepore Filter with Deposit of Taconite, Showing Difference Between Backed and Unbacked Areas

of this frit will permit very little filtration to occur, whereas the open areas will permit efficient filtration. The solution to this problem lies in the use of a backing filter. Figure 16 shows an optical micrograph of a Nuclepore filter with, as before, a heavy deposit of taconite tailings, in which only half of the Nuclepore filter was provided with a backing filter. The comparison of the unbacked and backed areas is striking. However, even when a backing filter is used, non-uniformities in the deposit are still found and these appear to be related to the fact that the Nuclepore filter is basically a hydrophobic material. The manufacturer applies a detergent to the surface of the filter, in order to render it hydrophilic; this process, however, does not appear to be entirely satisfactory in some batches. The plasma asher can be used to render the surfaces of many materials hydrophilic. Some success has been obtained in achieving a more uniform particulate deposit by a pretreatment of the Nuclepore filter in the plasma asher. The treatment used was approximately 10 seconds in the asher at a very low power of about 10 watts. A significant improvement in the uniformity of deposit was achieved, although some areas appeared to be resistant to this treatment. Figure 17 shows a comparison of treated and untreated filters, in their response to a heavy deposit of taconite. It can be seen that the islands of sparse deposit are not present on the treated filter.

It can now be seen that water filtration is not the simple topic it at first appears, if a uniform deposit of material on the filter is required. The problems can be largely overcome by bulk ordering of filters, specified with separators of polypropylene, rather than the usual paper variety to which release agents are sometimes applied. If a backing filter is also used, problems of non-uniformity can be minimized. However, problems still occasionally appear; some possibly caused by the filter clamping arrangements on the commercially available equipment, and some by localized hydrophobic areas on the filters themselves. Figure 18 shows an optical micrograph of a highly colored particulate deposit on a Nuclepore filter suffering from this particular problem. The effect is characterized by elongated oval shaped areas, having no particulate deposit at all. The filters concerned appear to be hydrophobic in these areas, since no filtration has occurred within them.

From the above discussion, it can be seen that filtration in the case of the Nuclepore is an extremely critical step, and the following instructions must be followed precisely if a uniform deposit is to be obtained. Initially, all equipment must be dry; partial wetting of the backing filters leads to a clogged situation which varies the filtration rate over the area of the filter. A conventional water aspirator may be used as the vacuum source, yielding a pressure of about 2kPa. Filtration rates are usually slow. The backing filter to be used may be any medium or large pore size Millipore filter; the 0.45 μm to 5 μm pore sizes have been used satisfactorily. The backing filter is placed onto the glass frit support with the vacuum turned on. The Nuclepore filter, shiny side up, is then placed on top of the backing filter. The suction permits the filters to settle firmly onto their support. If any folds appear in the Nuclepore filter it should be rejected and replaced. The liquid reservoir should then be clamped in position, keeping the vacuum on. The water sample should then be poured directly on to the Nuclepore and allowed to filter. If the reservoir is not large enough to contain the required volume, the additional liquid should be carefully added



Treated

Untreated

Fig. 17 Optical Micrograph of Nuclepore Filter with Deposit of Taconite, Showing Comparison of Untreated and Plasma Treated Filters

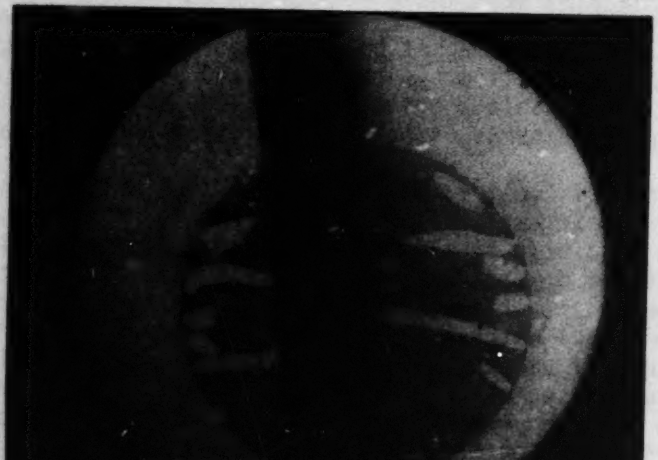


Fig. 18 Optical Micrograph of Nuclepore Filter with Deposit of Particulate, Showing Hydrophobic Areas of Filter which Permitted no Liquid to pass through

well before completion of filtration. After filtration, the sides of the funnel should not be rinsed. The Nuclepore filter should then be removed and dried. Chatfield and Glass (20) reported use of a Polyvic backing filter, in their ashing technique. This particular filter was selected because of its resistance to ethyl alcohol, the redispersal medium for the ash. This later work has shown that the non-uniformity problems experienced in the development of their method were, in fact, a consequence of the filter properties, rather than of the dispersion itself. A hydrophilic backing filter, such as a normal cellulose ester membrane, is now preferred, together with water as the redispersal medium.

SECTION 7

SAMPLE PREPARATION AND ANALYSIS

For all of this work the design of Jaffe washer shown in Figure 3 was used, although other work has shown that the washer described by Kalmus is simpler and easier to use, and also appears not to affect the results obtained.

7.1 CARBON COATED NUCLEPORE PREPARATION

For this preparation technique a portion of the Nuclepore filter is carbon-coated by vacuum evaporation. The distance between the filter and the carbon arc should be about 10 cm. During the evaporation the sample is rotated and tilted at various angles to ensure efficient coverage by carbon of all features on the filter. It is most important that the Nuclepore filter not be overheated; if the surface of the filter is exposed to excessive heat during the evaporation process the filter plastic exhibits cross-linking and becomes insoluble in chloroform. Such a filter gives an unsatisfactory electron microscope sample, since there is always undissolved plastic remaining after solvent extraction. The thickness of carbon to be used is found by experience; if it is too thin the replica will be fragile and breakage will occur, if it is too thick there will be lack of image contrast and interference with the diffraction process. Initially, problems were experienced in handling the Nuclepore filters. Attempts were made to dissolve the Nuclepore filters in the Jaffe washer, the carbon-coated side being placed downwards in contact with the copper electron microscope grid. Exposure to the chloroform vapor invariably caused the filter segments to curl up into a scroll, leading to an unsatisfactory preparation. This effect could be eliminated by placing a drop of chloroform from a microliter pipette directly onto the surface of the filter before filling up the Jaffe washer with chloroform. It was not possible to produce satisfactory preparations if the carbon-coated side was placed downwards, unless the drop of chloroform was added. However, a more satisfactory technique was found, in which the carbon-coated Nuclepore was placed with the carbon face upwards on the copper grid. Using this technique, the Jaffe washer is first filled with solvent. The portion of filter is cut from the Nuclepore, using a scalpel blade, and placed onto a copper electron microscope grid with the carbon facing upwards. The entire assembly is picked up using a pair of fine tweezers and placed boldly onto the Jaffe washer. Using this method, no curling occurs and almost a 100% success rate is obtained in the sample preparation. The minimum time for filter dissolution is approximately seven hours, but more satisfactory preparations are obtained if the assembly is allowed to stand for up to 48 hours.

7.2 DIRECT DISSOLUTION OF UNCOATED NUCLEPORE ON CARBON-COATED GRIDS

In this preparation technique the uncoated Nuclepore filter is placed with its active side down on to a carbon-coated electron microscope grid. This assembly is placed in the Jaffe washer, prior to addition of the chloroform. A small drop of chloroform was applied to the filter from a microliter pipette, as in the case of the coated Nuclepore method, without which it was not possible to produce grids with undamaged carbon films. The preparation technique using the initial drop of chloroform appeared to be satisfactory, and samples were produced which contained no confusing replication detail. Attempts were made to use the condensation washer for this dissolution process, using chloroform as the solvent. After many experiments it was found that the condensation washer was extremely unstable when operated using chloroform as the solvent. In order to obtain condensation in the vicinity of the grids, it was necessary to operate the washer fairly close to the boiling point of chloroform. Unfortunately, explosive generation of vapor would occasionally occur, leading to total loss of the samples. The condensation washer technique using chloroform was therefore abandoned in favor of the more reliable and simple Jaffe washer.

7.3 JAFFE WASHER DISSOLUTION OF MILLIPORE FILTERS

Before adding acetone to the Jaffe washer, carbon-coated grids were placed on the wick, and portions of the Millipore to be processed were placed on them, the active filter surface facing downwards in contact with the carbon film. A 5 microliter drop of acetone was initially applied to each filter, after which the washer was charged with acetone. The washer was best left for a minimum period of 24 hours. Even after periods of 24 hours undissolved filter medium remained on the samples.

7.4 CONDENSATION WASHER DISSOLUTION OF 0.45 μm PORE SIZE MILLIPORE FILTERS

A substantial amount of effort was expended in investigating this technique. The condensation washer was operated under conditions as close to those of the published interim method (26) as possible. The brass holder designed by Millette (24) improved the ease of handling samples in this washer. Carbon-coated grids were placed in the brass holder, and portions of the Millipore filters to be processed were placed on them with the active surfaces facing downwards. A 5-10 microliter droplet of acetone was applied to each filter from a microliter pipette. The cold finger with the brass holder on it was then inserted into the washer, which was then switched on. Heating of the solvent reservoir was adjusted such that the condensation level of solvent on the interior of the equipment appeared to be approximately the level of the grids. Under these conditions washing action was very gentle and even after a period of 6 to 12 hours undissolved plastic was still observed. The degree of washing and the residual plastic found was comparable, therefore, with the treatment given in the Jaffe washer. In order to obtain stable operation of the condensation washer it was found necessary to control both the temperature and the flow of the cooling water in the cold finger. In addition, the washer required almost constant observation, since minor changes of water flow and temperature in the cold finger and also changes in supply voltage could cause variation in its operating characteristics. A large number of initial experiments were made to investigate the operation of the washer before any samples were processed. Thermocouples, placed in the vicinity of the samples, indicated that movement of the "condensation level" was a very sensitive function of the heat input to the solvent flask. However, the height of the condensation level on the interior of the glassware can only be an indication of the solvent action on the grids themselves. This will be controlled also by the cooling water temperature in the cold finger, and there appears to be no simple way to monitor the condensation rate on the grids, apart from counting drops as they fall back into the reservoir flask.

7.5 ONTARIO RESEARCH FOUNDATION ASHING TECHNIQUE

Although the original method was developed using a 0.1 μm pore size Millipore filter, in this particular program the 0.45 μm filters were used. It has been found in previous work that if the Millipore is ashed directly, some samples yield ash which is difficult to redisperse. Satisfactory ashing behavior is usually obtained if the filter is first dissolved in filtered acetone in a 50 ml beaker, one drop of distilled water added, and the acetone then evaporated to dryness. A white deposit remains in the bottom of the beaker. The deposit was ashed for a period of 24 hours under standard conditions, i.e. at a pressure of 1 Torr of pure oxygen and an indicated power of approximately 20 watts. About half way through the ashing cycle the power was increased to 40 watts so that complete oxidation could be assured. After ashing, 20 ml of water was added to the beaker, and the solid material was dispersed by ultrasonic treatment at a power of 0.5 watt/ml for 30 seconds. This dispersion was then filtered through a 0.1 μm pore size Nuclepore filter. The Nuclepore filter was then processed by the carbon-coated Nuclepore technique. The initial group of filters processed indicated that there was far more insoluble ash residues produced than had been observed previously. The function of the ashing procedure is to remove solid materials which interfere with the analysis; in fact this particular batch of Millipore filters yielded more solid material than the ashing procedure was intended to remove. The composition of the ash residue was investigated by energy dispersive X-ray spectrometry, and found to consist principally of calcium, sulphur and phosphorus. Discussions with Millipore Corporation revealed that detergents are added to the surface of the Millipore filters to assist filtration properties, and that the origin of the sulphur and phosphorus was from these detergents. However, the origin of the calcium is a present uncertain. What is certain is that the addition of water to this mixture of chemical radicals will yield insoluble calcium phosphate and calcium sulphate. To overcome this problem, the redispersal medium was changed from distilled water to a 0.01% solution of the disodium salt of ethylenediamine tetraacetic acid. This material complexes all free calcium, and inhibits the formation of insoluble precipitates of calcium sulphate and calcium phosphate. The procedure was found entirely satisfactory, and very little residual ash was found on the Nuclepore filters when this redispersal technique was in use. There was also no evidence of any adverse affect on the asbestos fibers contained. However, as in the case of all other reagents used in the preparation of the samples for asbestos counting, the EDTA solution was filtered through a 0.1 μm Nuclepore filter prior to use. Examination of the Nuclepore filter used for this reagent filtration indicated that there was significant contamination of the crystalline EDTA salt by chrysotile asbestos.

7.6 FIBER COUNTING

For each sample several specimen grids were prepared. A total of 10 grid openings from the available specimen grids were examined for the presence of fibers. Grids were counted at a magnification of about 20,000 - 25,000, and the actual grid opening dimensions were measured at a magnification of approximately 2,000. Since the dimensions of the grid openings have been observed to vary significantly within an individual grid, it is the practise to count and measure all fibers within the grid opening, and then at a reduced magnification to measure the dimensions of the grid opening so that the error due to this effect can be eliminated. Wherever possible, fibers touching the grid bars were counted on two sides of the opening only. In these cases their lengths cannot be accurately stated. However, the grid loadings were generally low, and such instances were rare. Fibers were placed into the three categories previously discussed, i.e. confirmed amphibole, probable amphibole, and chrysotile. Where fibers, particularly amphibole fibers, were too large to identify by electron diffraction, a search was made around the edge of the particle in order to find suitable areas which would give SAED patterns. In most cases this could be achieved. The fiber counts obtained were processed by a computer program which performed the relevant statistical analyses and also computed the fiber number and mass concentrations and their size distributions.

SECTION 8

STATISTICAL TREATMENT OF RESULTS

8.1 UNIFORMITY OF DEPOSIT ON THE ELECTRON MICROSCOPE GRIDS

A check was made using the chi-squared test, to determine whether the number of fibers found on individual grid squares were randomly and uniformly distributed among the grid squares. If the total number of fibers found in k grid squares is n, and the areas of the k individual grid squares are designated A_i to A_k, then the total area examined

$$A = \sum_{i=1}^{k} A_i$$

The fraction of the total area represented by the individual grid square area, p_i, is given by A_i/A. If the fibers are randomly and uniformly dispersed over the k grid squares counted, the expected number of fibers falling in the region of one grid square with area $A_i = np_i$. If the observed number found in that grid square is n_i, then

$$\chi^2 = \sum_{i=1}^{k} \frac{(n_i - np_i)^2}{np_i}$$

This value is compared with the significance point of the χ^2 distribution, having (k - 1) degrees of freedom. We may express our reluctance to discard the idea that the deposit is uniform by establishing a very low value of α, the significance level, and in this work a significance level α of 0.1% has been used. The use of such a loose criterion for uniformity permits an economic and realistic proportion of the samples to be considered acceptable.

8.2 THE BEST ESTIMATE AND CONFIDENCE INTERVAL OF THE FIBER CONCENTRATION

In the fiber analysis we wish to sample about 10 grid openings from the population of grid openings and determine the mean grid opening fiber count for the population on the basis of our sample. We also wish to determine the interval about the sample mean, which, with a stated degree of confidence, will contain the population mean. This is achieved by calculating the simple arithmetic mean, followed by computation of a confidence interval using the

Student "t" distribution. For the two-sided "t" test, n values of grid square fiber count are used. The sample estimate of variance s^2 is first calculated, where

$$s^2 = \frac{n \sum_{i=1}^{k} X_i^2 - \left\{ \sum_{i=1}^{n} X_i \right\}^2}{n\,(n-1)}$$

If the desired confidence is 100 $(1 - \alpha)$%, for the two-sided interval the value of $t = t_{1-\alpha/2}$ is obtained for (n - 1) degrees of freedom. For example if the desired confidence level is 95%, for the two-sided interval the value of $t_{0.975}$ is obtained for (n - 1) degrees of freedom. If the mean value of fiber concentration is calculated to be $\overline{X}$, the upper and lower values of the confidence interval are given by

$$X_u = \overline{X} + \frac{ts}{\sqrt{n}}$$

and $$X_L = \overline{X} - \frac{ts}{\sqrt{n}}$$

This confidence interval is the range of values within which, with a stated degree of confidence, the mean value of all grid squares may be expected to lie. It is important to recognize that the chi-squared test and the calculation of the confidence interval are not the same procedure. The chi-squared test is the appropriate test to demonstrate that the fibers are randomly and uniformly distributed on the grid squares selected. A very lossy preparation, for example, which has lost all the fibers from the specimen grid except one, all other grid squares containing no fibers, will give a very low value of chi-squared. This is a statement that those fibers present are very uniformly dispersed, i.e. a nearly constant zero. However, the 95% confidence interval of such a preparation would be very large, indicating an imprecise result.

8.3 STATISTICAL SIGNIFICANCE OF LOSS MEASUREMENTS

Using this procedure a one sided test is used to determine if the mean value for measurements by one technique significantly exceeds the mean value for measurements using another technique.

Initially, the means and sample estimates of variance are calculated for the two techniques. If $\overline{X}_A$ and $\overline{X}_B$ are the means for the two techniques and s^2_A and s^2_B are the sample estimates of variance for n_A and n_B measurements respectively, the estimated variances of $\overline{X}_A$ and $\overline{X}_B$ are $V_A = s^2_A/n_A$ and $V_B = s^2_B/n_B$.

The effective number of degrees of freedom is f, where

$$f = \frac{\left(V_A + V_B\right)^2}{\frac{V_A{}^2}{(n_A + 1)} + \frac{V_B{}^2}{(n_B + 1)}} - 2$$

If the significance level of the test is α, then the value of $t_{(1 - \alpha)}$ is obtained for f' degrees of freedom, where f' is the nearest integer to f.

The value of $u = t_{(1 - \alpha)} \cdot \sqrt{V_A + V_B}$ is obtained, and this is compared with the difference in the means $(\overline{X}_A - \overline{X}_B)$. If $u > (\overline{X}_A - \overline{X}_B)$ there is no reason to believe that $\overline{X}_A$ exceeds $\overline{X}_B$ at the stated level of significance.

8.4 VARIABILITY WITHIN TECHNIQUES

Within a particular technique, the spread of results obtained may be different from the spread of results within another technique. The variability of a particular technique can be expressed using the relative standard deviation, of coefficient of variation, i.e. the standard deviation of the results divided by their mean value.

SECTION 9

RESULTS

Tables A-1 to A-23 in the Appendix summarize results obtained for all samples analyzed. For each sample, the sequence number in the filtration series is stated, followed by the mean fiber concentration, the 95% confidence interval, and the mass concentration calculated by summation of the volume of fibers. The grid distribution is expressed as a value of chi-squared, together with the significance level at which grid uniformity could be accepted. Table A-22 shows the chrysotile results as measured by the ashing technique. Examination of the crocidolite and taconite results processed by the ashing technique showed that the 0.45 μm Millipore filters were contaminated by chrysotile. The chrysotile fiber counts obtained on the ashed crocidolite samples are reported in Table A-23. The significance of this filter contamination on the results is discussed later.

In Tables A-1 to A-21, it can be seen immediately that the variability of the results within a particular method is highest for the direct transfer Millipore techniques. Figures 19-25 show the fiber length distributions for the various categories of reported fibers. Examination of the size distributions for total fibers indicates that there is very little distortion of the size distributions by the individual methods of sample preparation. The size distribution in each case is obtained by summing the individual distributions for all samples within any one technique. Each size distribution plotted, therefore, represents a minimum of 10 individual measurements. Tables 1-5 show summaries of the experimental results. Table 1, for example, shows the mean value of the fiber concentration for 26 separate preparations of chrysotile filters by the carbon-coated Nuclepore technique. The mean values of the technique results are given together with their standard deviations. The data for the ORF ashing technique have been corrected for the observed chrysotile contamination of the Millipore filters, using the mean chrysotile count observed on the crocidolite filters as a means of establishing the contamination level. As the best available expedient, the mean chrysotile count obtained on these filters was subtracted from each individual chrysotile result.

It should also be mentioned that the computed mass concentrations quoted are presented as an indication of the reproducibility of this measurement. Although the values obtained are not sufficiently accurate for their use in loss calculations, it does appear that a useful estimate of mass concentration can be made.

Figure 19 Fiber Length Distribution, Chrysotile

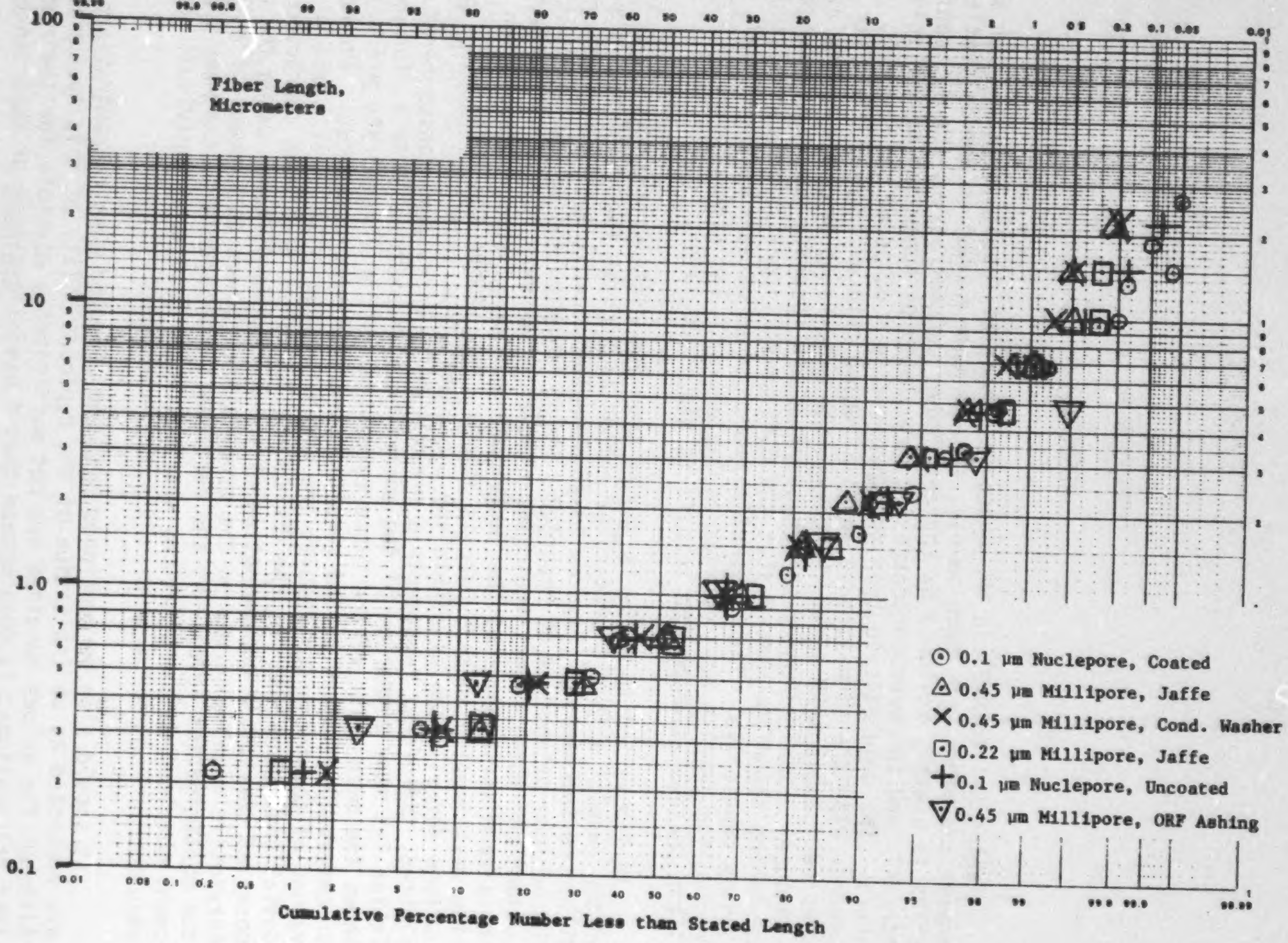


Figure 20 **Fiber Length Distribution, Confirmed Crocidolite**

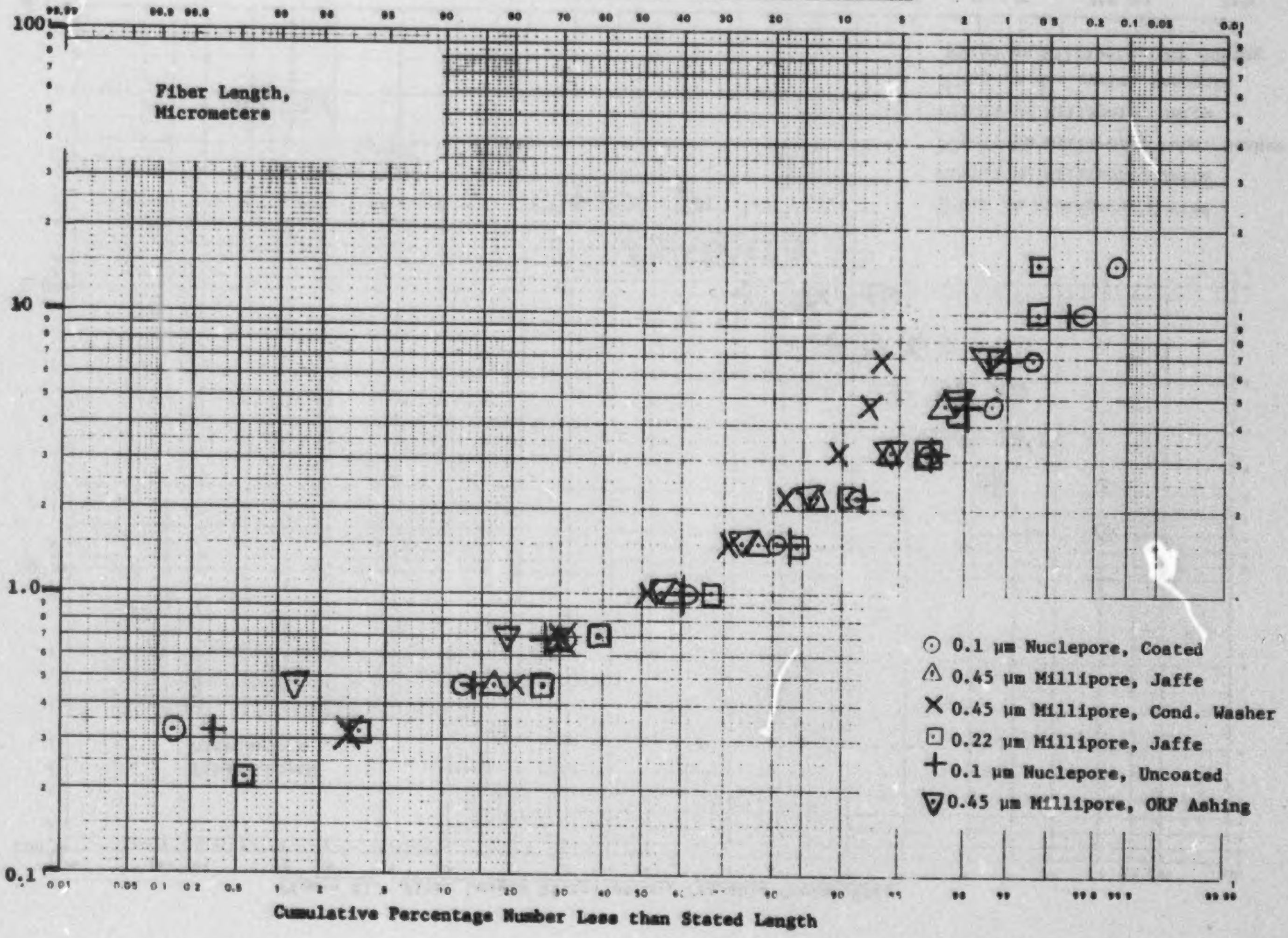


Figure 21. **Fiber Length Distribution, Probable Crocidolite**

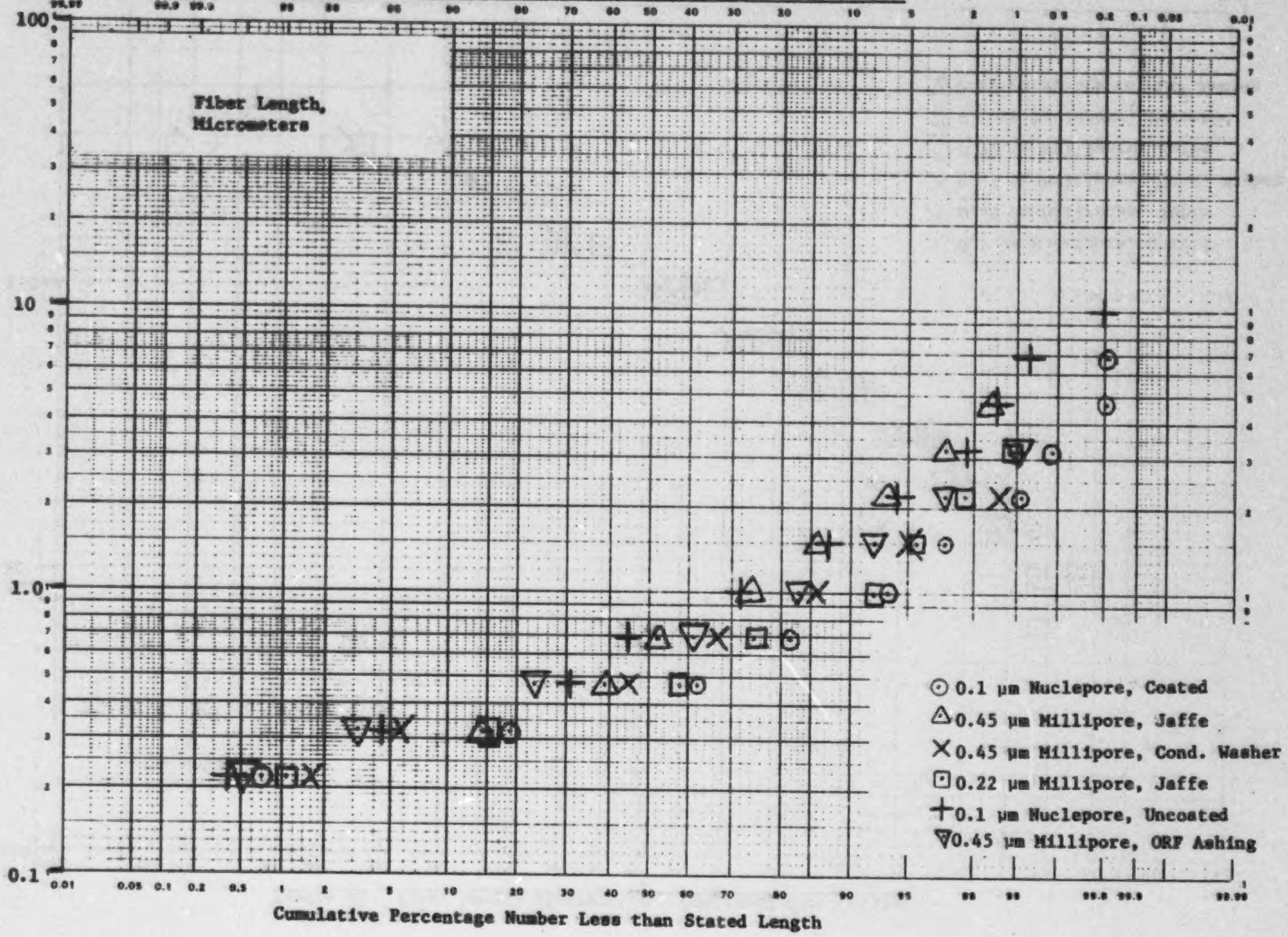


Figure 22. Fiber Length Distribution, Total Crocidolite Fibers

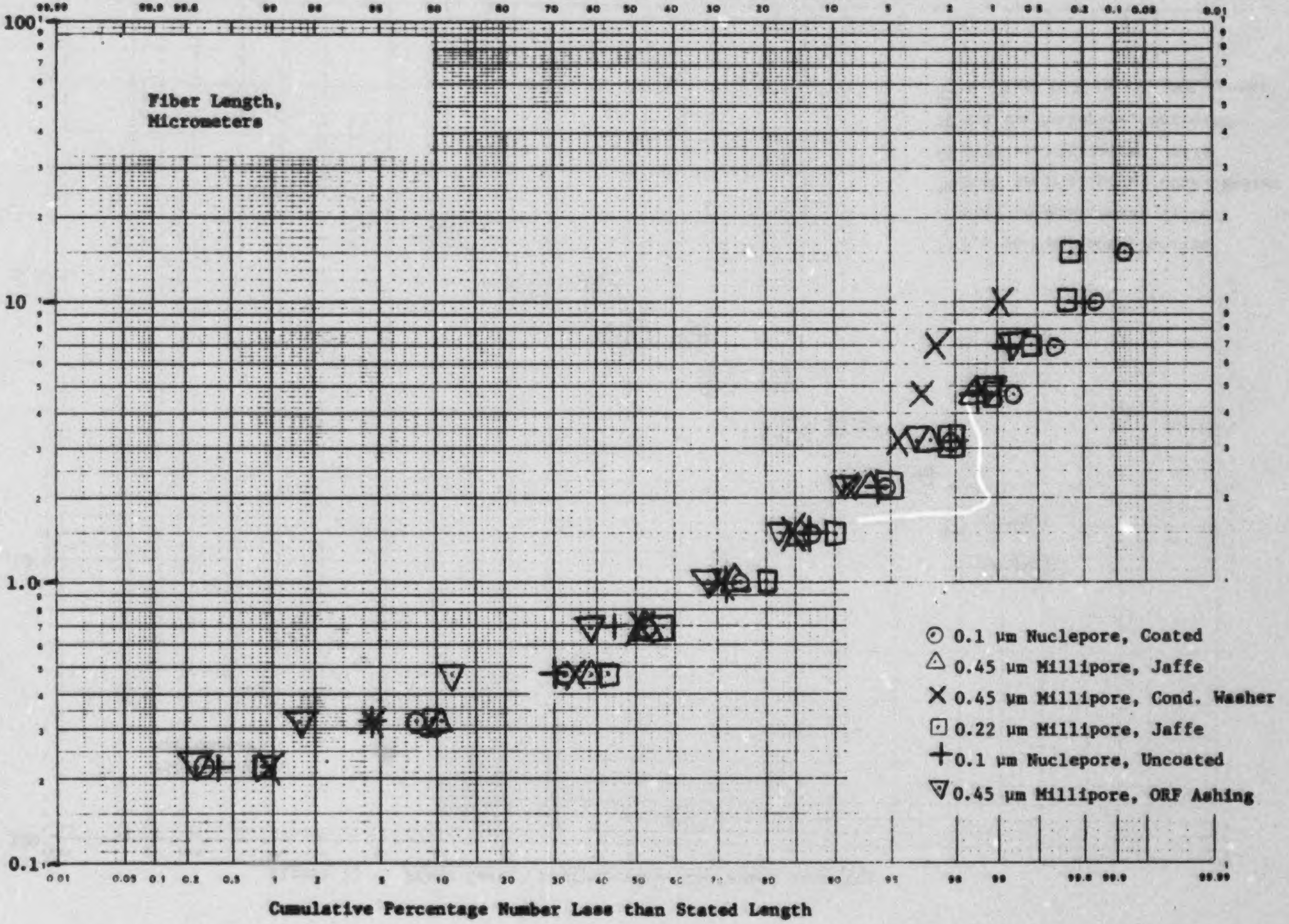


Figure 23 Fiber Length Distribution, Confirmed Taconite

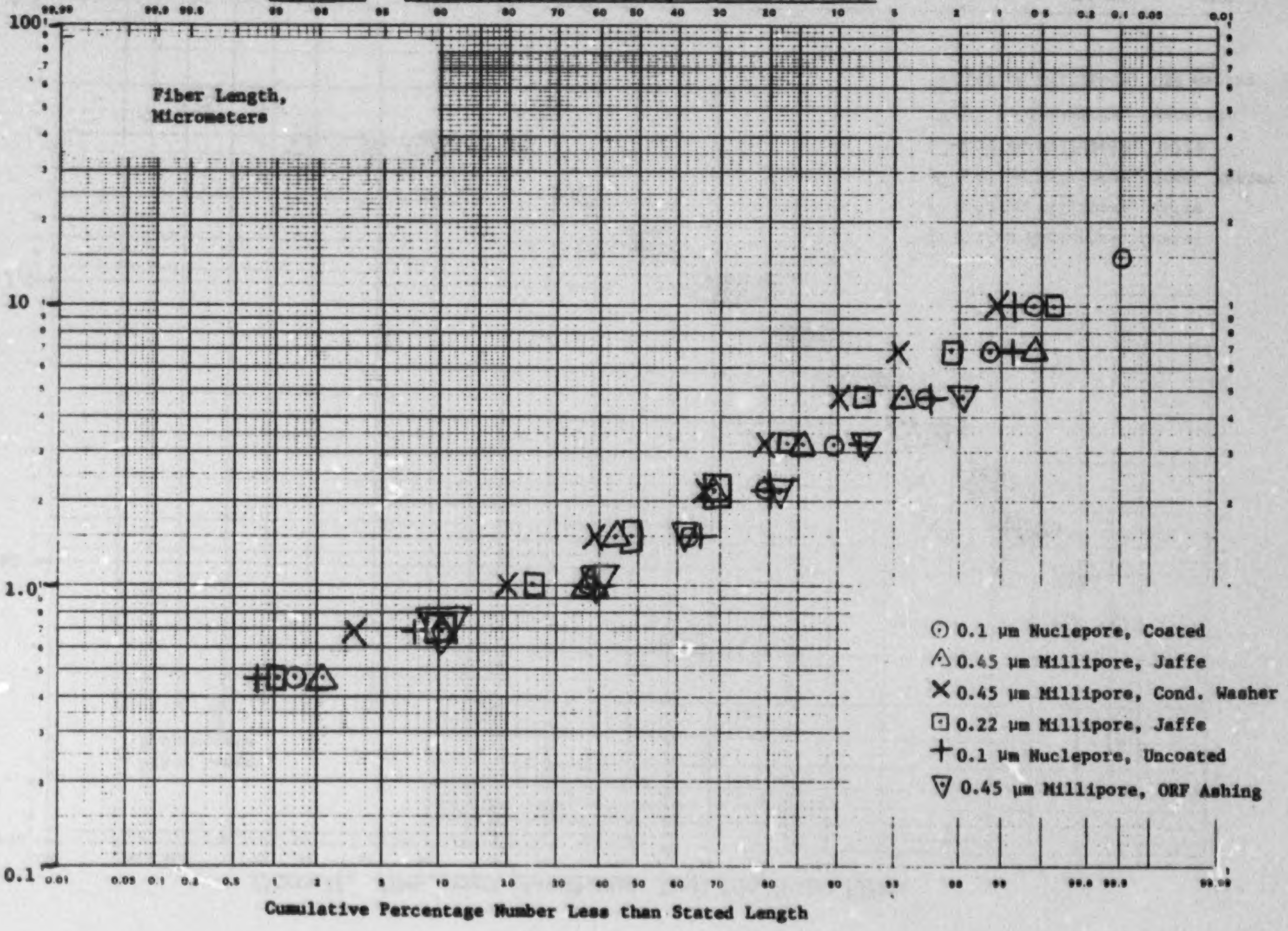


Figure 24 Fiber Length Distribution, Probable Taconite

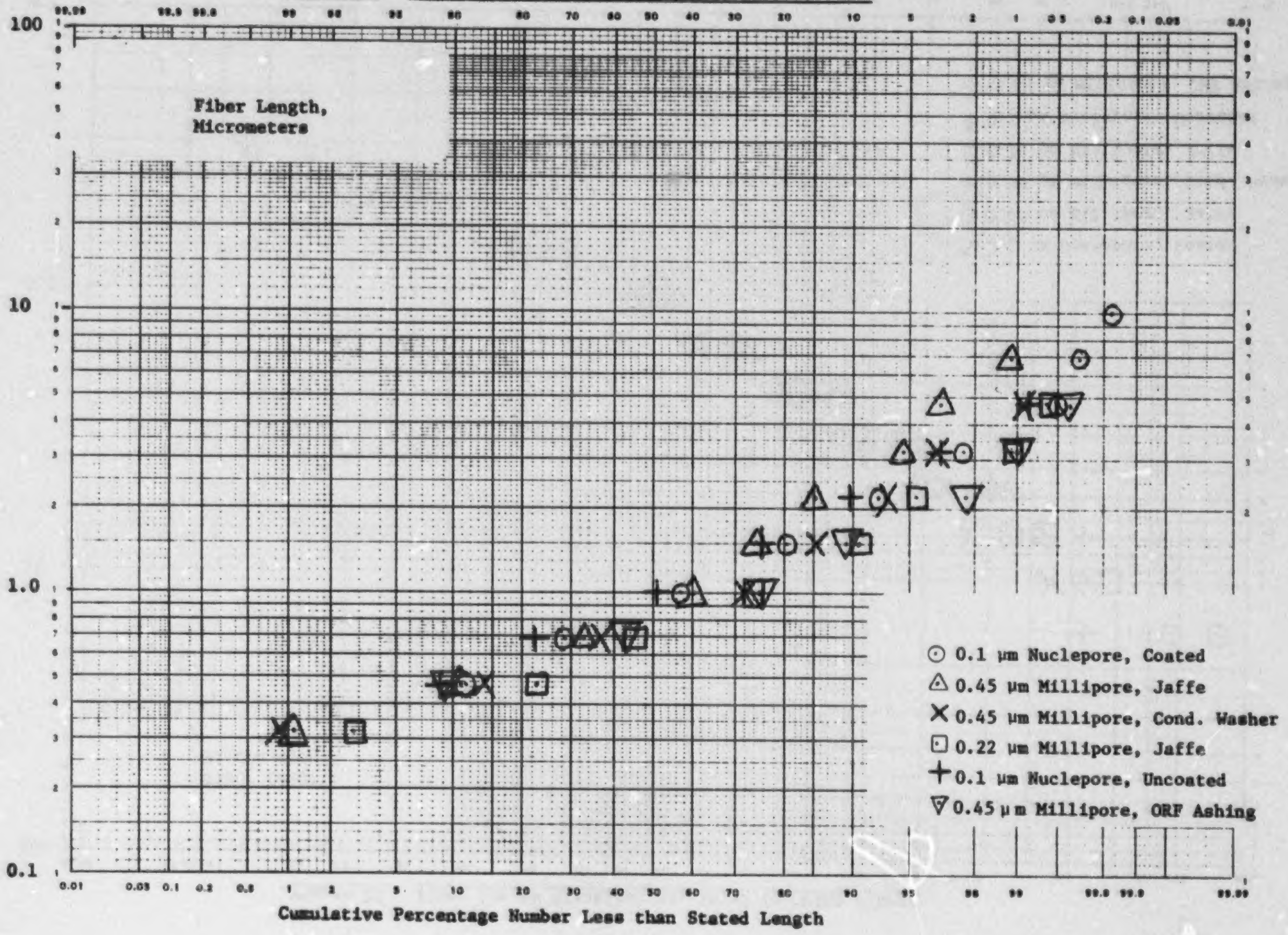


Figure 25 **Fiber Length Distribution, Total Taconite Fibers**

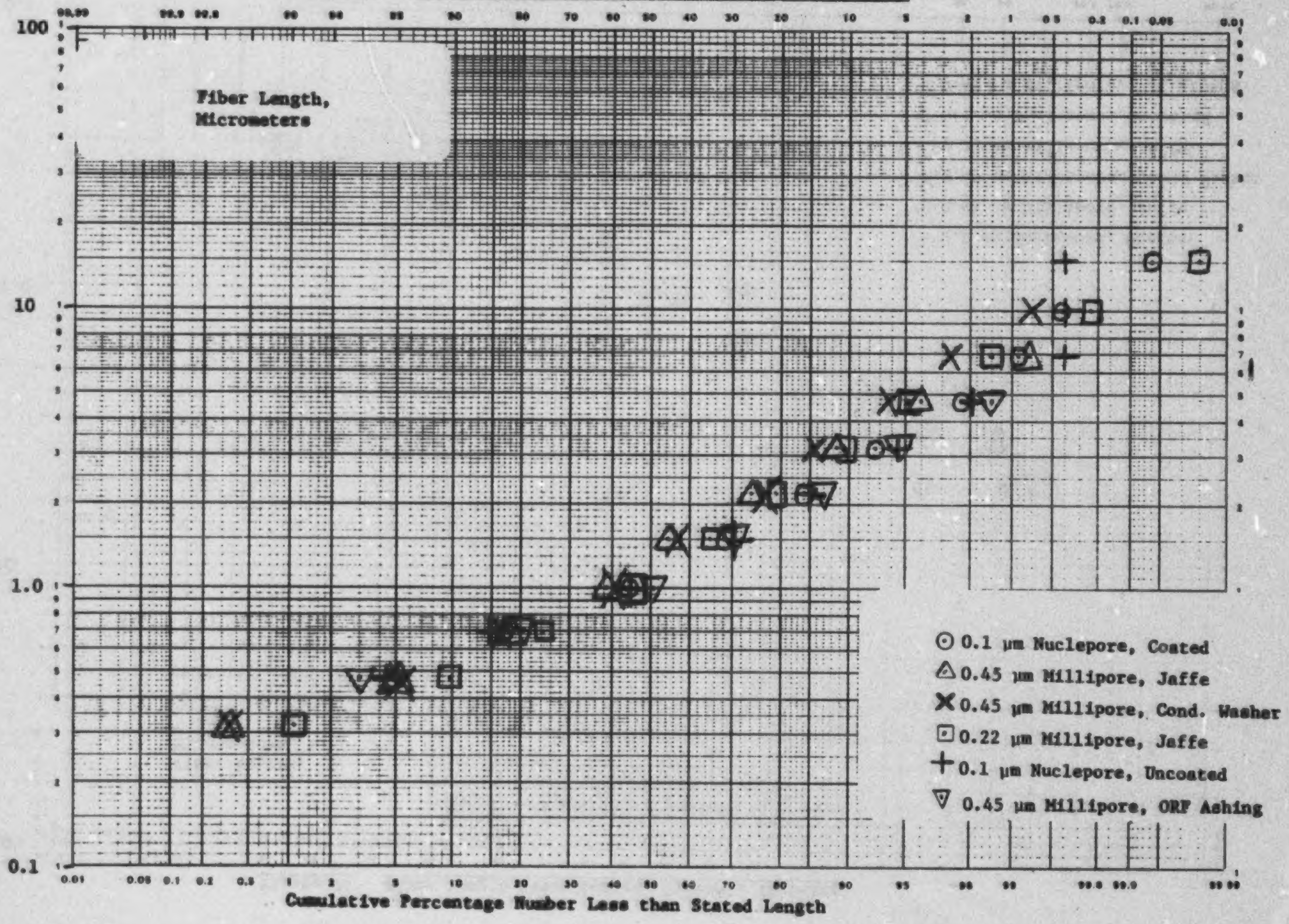


TABLE 1

Summary of Experimental Results: Chrysotile

Numerical Concentrations

(Values in 10^6 Fibers/Liter)

Preparation Technique	Number of Samples Analysed	Concentration of Chrysotile x 10^6 Fibers/liter		Mass Concentration of Chrysotile, μg/liter	
		Mean Value	Standard Deviation	Mean Value	Standard Deviation
Carbon Coated Nuclepore	26	23.42	8.58	0.320	0.227
Uncoated Nuclepore Jaffé Wick	12	18.52	7.34	0.246	0.144
0.45μm Millipore Jaffé Wick	14	4.71	3.66	0.063	0.057
0.45μm Millipore Condensation Washer	12	9.62	5.79	0.113	0.069
0.22μm Millipore Jaffé Wick	11	10.02	3.32	0.167	0.137
ORF Ashing Technique	10	25.28	9.67	0.234	0.072

TABLE 2

Summary of Experimental Results: Crocidolite

Numerical Concentrations

(Values in 10^6 Fibers/Liter)

Preparation Technique	Number of Samples Analysed	Confirmed Amphibole		Probable Amphibole		Total Fibers		Proportion Yielding Identified SAED Patterns, %
		Mean Count	Standard Deviation	Mean Count	Standard Deviation	Mean Count	Standard Deviation	
Carbon Coated Nuclepore	20	8.11	2.88	5.81	1.86	13.92	3.24	58.3
Uncoated Nuclepore, Jaffé Wick	10	5.64	2.42	3.74	1.28	9.38	3.48	60.1
0.45μm Millipore Jaffé Wick	10	1.50	0.96	1.37	0.78	2.88	1.59	52.1
0.45μm Millipore Condensation Washer	14	1.85	1.60	2.13	1.74	3.98	3.17	46.5
0.22μm Millipore Jaffé Wick	13	3.31	2.49	3.45	2.35	6.75	4.70	49.0
ORF Ashing Technique	10	4.39	0.95	3.85	0.66	8.24	1.36	53.3

TABLE 3

Summary of Experimental Results: Crocidolite

Mass Concentrations

(Values in micrograms/liter)

Preparation Technique	Number of Samples Analysed	Confirmed Amphibole		Probable Amphibole		Total Amphibole Mass		Proportion of Mass which Yielded SAED Patterns, %
		Mean Mass Conc.	Standard Deviation	Mean Mass Conc.	Standard Deviation	Mean Mass Conc.	Standard Deviation	
Carbon Coated Nuclepore	20	1.294	0.694	0.155	0.121	1.449	0.690	89.3
Uncoated Nuclepore Jaffé Wick	10	0.626	0.318	0.093	0.085	0.719	0.331	87.1
0.45μm Millipore Jaffé Wick	10	0.192	0.230	0.032	0.023	0.224	0.234	85.7
0.45μm Millipore Condensation Washer	14	0.410	0.438	0.068	0.104	0.478	0.487	85.8
0.22μm Millipore Jaffé Wick	13	0.331	0.206	0.075	0.065	0.406	0.232	81.5
ORF Ashing Technique	10	0.761	0.324	0.144	0.055	0.904	0.338	84.2

TABLE 4

Summary of Experimental Results: Taconite

Numerical Concentrations

(Values in 10^6 Fibers/Liter)

Preparation Technique	Number of Samples Analysed	Confirmed Amphibole		Probable Amphibole		Total Fibers		Proportion Yielding Identified SAED Patterns, %
		Mean Count	Standard Deviation	Mean Count	Standard Deviation	Mean Count	Standard Deviation	
Carbon Coated Nuclepore	20	16.26	3.86	8.71	3.11	24.96	5.69	65.1
Uncoated Nuclepore Jaffé Wick	10	25.36	5.26	15.9	1.52	40.75	5.22	61.0
0.45μm Millipore Jaffé Wick	10	5.24	1.42	2.66	1.11	7.90	2.28	66.3
0.45μm Millipore Condensation Washer	10	5.45	2.84	3.48	1.42	8.92	4.08	61.1
0.22μm Millipore Jaffé Wick	10	7.65	2.68	4.91	1.94	12.56	4.09	60.9
ORF Ashing Technique	10	22.34	2.46	10.15	2.70	32.49	4.26	68.6

TABLE 5

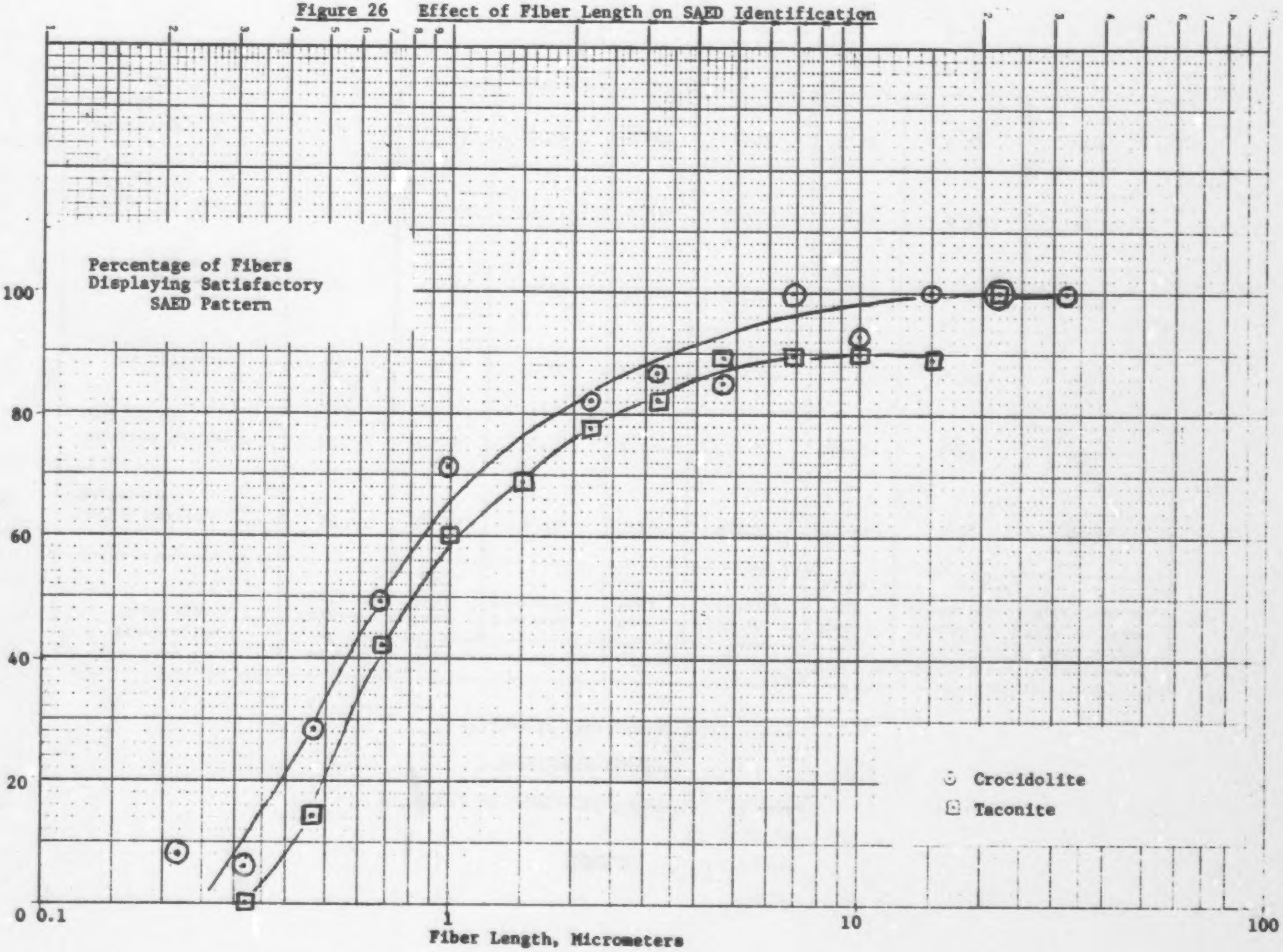
Summary of Experimental Results: Taconite

Mass Concentrations

(Values in micrograms/liter)

Preparation Technique	Number of Samples Analysed	Confirmed Amphibole		Probable Amphibole		Total Amphibole Mass		Proportion of Mass which Yielded SAED Patterns, %
		Mean Mass Conc.	Standard Deviation	Mean Mass Conc.	Standard Deviation	Mean Mass Conc.	Standard Deviation	
Carbon Coated Nuclepore	20	10.29	6.78	0.194	0.162	10.49	6.83	98.1
Uncoated Nuclepore Jaffé Wick	10	10.22	8.45	0.148	0.048	10.36	8.48	98.1
0.45μm Millipore Jaffé Wick	10	3.82	2.62	0.048	0.039	3.87	2.63	98.7
0.45μm Millipore Condensation Washer	10	5.21	4.64	0.034	0.013	5.24	4.63	99.4
0.22μm Millipore Jaffé Wick	10	8.26	6.21	0.031	0.015	8.29	6.21	99.6
ORF Ashing Technique	10	14.35	6.02	0.169	0.064	14.52	6.02	98.8

Figure 26 Effect of Fiber Length on SAED Identification



9.1 CONTAMINATION OF 0.45 μm MILLIPORE FILTERS BY CHRYSOTILE

At this point some remarks about the significance of the chrysotile contamination should be made. The normal practise when using the ORF ashing technique is to remove silicate contamination, fibrous or otherwise, by washing the Millipore filters in concentrated hydrofluoric acid prior to use. On this occasion, this was not done because of possible deleterious effects of the treatment on the solubility of the filters in acetone and consequent interference with the direct dissolution methods. Previously reported (27) chrysotile contamination of filters was sufficiently low that it would be of little concern in this study. However, since the mean contamination level was equivalent to a result of 12.72 x 10^6 fibers/l, it is interesting to speculate on the reasons why it was not observed at these levels on the Millipore dissolution methods. The reason would appear to be that the efficiency of particle transfer to the grid during the dissolution methods is a function of the depth of the fibers within the open filter structure. This conclusion is supported by the fact that in Tables 1, 2 and 4 it can be seen that the 0.22 μm pore size Millipore gave a higher result on every occasion than the 0.45 μm Millipore, in which the intentional fibers would be more deeply buried in its structure. Thus during the filter dissolution process, most of the uniformly distributed contamination fibers in the depth of the filter were probably lost, whereas they were efficiently collected by the ORF ashing procedure to be deposited on the final replica.

9.2 IDENTIFICATION OF AMPHIBOLES BY SAED

In Table 2 it can be seen that the proportion of particles yielding identified SAED patterns is remarkably constant between individual preparation techniques, the actual proportion varying from 49% to 60%. On the other hand, in Table 3 it can be seen that the majority of the mass is in the identified category. The same conclusion can be drawn for taconite, in which the identified numerical proportion is between 51% and 69% of the total. Figure 26 shows the success of identification by SAED as a function of fiber length, for both crocidolite and taconite. It can be seen that the chance of positive identification falls markedly for fibers below 1 μm in length, whereas above about 4 μm to the maximum size studied, i.e. 32 μm, nearly all the fibers fall within the identified category.

9.3 DISCUSSION OF FIBER LOSSES AND REPRODUCIBILITY

The principal results of the study are shown in Tables 6-8, in which fiber losses and variability analyses are tabulated for the three asbestos materials and all of the preparation methods. In these tables the carbon-coated Nuclepore preparation is assumed to have negligible losses, and the recoveries of the other methods are referred to it. For each preparation method, the question of whether the declared loss is detectable at 5% significance is asked. This test is necessary in view of the large standard deviations of the results obtained by some methods. Durther evidence regarding the negligible losses of the coated Nuclepore preparation is presented later in this section.

TABLE 6

Fiber Loss and Variability Analysis: Chrysotile

Preparation Technique	Mean Value, x 10^6 Fibers/1	95% Confidence Interval, x 10^6 Fibers/liter	Number of Filters Analyzed	Std.Dev. of Mean, x 10^6 Fibers/1	Loss * in Preparation, %	Is the Numerical loss detectable at 5% Significance?	Significance Level Required to Reverse Decision, %	Coeff. of Variation $\left(\frac{\text{Std. Dev.}}{\text{Mean}}\right)$
Carbon Coated Nuclepore	23.42	19.95 - 26.89	26	8.58	-	-	-	0.366
Uncoated Nuclepore Jaffé Wick	18.52	13.86 - 23.18	12	7.34	21	Yes	2.5	0.396
0.45μm Millipore Jaffé Wick	4.71	2.59 - 6.82	14	3.66	80	Yes	<0.5	0.777
0.45μm Millipore Condensation Washer	9.62	5.94 - 13.30	12	5.79	59	Yes	<0.5	0.602
0.22μm Millipore Jaffé Wick	10.02	7.79 - 12.25	11	3.32	57	Yes	<0.5	0.331
ORF Ashing Technique	25.28	18.36 - 32.20	10	9.67	0	No	30	0.383

* Losses are referred to Coated Nuclepore Technique Values

TABLE 7

Fiber Loss and Variability Analysis: Crocidolite

Preparation Technique	Mean Value, x 10^6 Fibers/l	95% Confidence Interval, x 10^6 Fibers/liter	Number of Filters Analyzed	Std.Dev. of Mean, x 10^6 Fibers/l	Loss * in Preparation %	Is the Numerical loss detectable at 5% Significance?	Significance Level Required to Reverse Decision, %	Coeff. of Variation $\frac{\text{(Std. Dev.)}}{\text{Mean}}$
Carbon Coated Nuclepore	13.92	12.40 - 15.44	20	3.24	-	-	-	0.233
Uncoated Nuclepore Jaffé Wick	9.38	6.89 - 11.87	10	3.48	33	Yes	<0.5	0.371
0.45μm Millipore Jaffé Wick	2.88	1.74 - 4.02	10	1.59	79	Yes	<0.5	0.552
0.45μm Millipore Condensation Washer	3.98	2.15 - 5.81	14	3.17	71	Yes	<0.5	0.796
0.22μm Millipore Jaffé Wick	6.75	3.91 - 9.59	13	4.70	52	Yes	<0.5	0.696
ORF Ashing Technique	8.24	7.27 - 9.21	10	1.36	41	Yes	<0.5	0.165

* Losses are referred to Coated Nuclepore Technique Values

TABLE 8

Fiber Loss and Variability Analysis: Taconite

Preparation Technique	Mean Value, x 10^6 Fibers/1	95% Confidence Interval, x 10^6 Fibers/liter	Number of Filters Analyzed	Std.Dev. of Mean, x 10^6 Fibers/1	Loss * in Preparation %	Is the Numerical loss detectable at 5% Significance?	Significance Level Required to Reverse Decision, %	Coeff. of Variation $\frac{\text{(Std. Dev.)}}{\text{Mean}}$
Carbon Coated Nuclepore	24.96	22.30 - 27.62	20	5.69	-	-	-	0.228
Uncoated Nuclepore Jaffé Wick	40.75	36.80 - 44.68	10	5.22	0^+	No	+	0.128
0.45μm Millipore Jaffé Wick	7.90	6.27 - 9.53	10	2.28	68	Yes	<0.5	0.289
0.45μm Millipore Condensation Washer	8.92	6.00 - 11.84	10	4.08	64	Yes	<0.5	0.457
0.22μm Millipore Jaffé Wick	12.56	9.63 - 15.49	10	4.09	50	Yes	<0.5	0.326
ORF Ashing Technique	32.49	29.44 - 35.54	10	4.26	0	No	+	0.131

* Losses are referred to Coated Nuclepore Technique Values

+ Result Higher than that for Coated Nuclepore Technique

9.3.1 Chrysotile

In Table 6 it can be seen that for chrysotile, all of the Millipore dissolution techniques give large and statistically significant losses relative to the coated Nuclepore technique, although the losses of the uncoated Nuclepore method were less significant and indeed become insignificant at the 2.5% level. This is further evidence for the conclusion that the efficiency of particle transfer is related to its initial distance in the filter structure from the carbon film substrate. In the case of the Nuclepore, all of the particulate is at its surface and efficient direct transfer appears to take place. In the case of chrysotile, no statistically significant fiber loss was observed when the samples were prepared by the ORF ashing technique. The variability can be considered in terms of particle movement during dissolution. The high values of this parameter, indicating a wide spectrum of results, occur principally in connection with the Millipore dissolution methods, although the 0.22 μm Millipore is an exception to this rule. If it is recalled that the filters were all of identical, or at least very similar fiber loadings, the variability should be constant if there is no contribution to it from the preparation technique. It is interesting that the variability of the coated Nuclepore technique using ashing (ORF ashing technique), is very nearly equal to that for the direct coating technique, indicating that the ashing step does not increase the range of results obtained.

9.3.2 Crocidolite

Table 7 summarizes the results for crocidolite. As in the case of chrysotile, the largest losses and the highest variabilities were displayed by the Millipore direct transfer technique, notably those using the 0.45 μm Millipore. The uncoated Nuclepore technique yielded a 33% mean loss, with an increased variability. Although the ashing technique gave a 41% mean loss, the variability of the results was smaller than that for any other technique, reflecting the more complete dispersion of particles obtained when ultrasonic treatment is used.

9.3.3 Taconite

In Table 8 the results for taconite show generally the same pattern, in that the high losses were observed for the Millipore direct dissolution preparations. The variabilities were also, as before, highest for these techniques. However, both the ORF ashing technique and the direct dissolution Nuclepore technique yielded higher results on this occasion than the direct carbon-coated preparation. The reason perhaps lies in more efficient dispersal of fibers by these methods, either by the action of ultrasonic energy in the one case, or chloroform during the dissolution step in the other. Fibers which would normally remain aggregated thus become available for inspection as individual fibers, increasing the numerical result. This is confirmed by observations on specimens of taconite prepared by the carbon-coated Nuclepore technique, which indicate that a significant proportion of the material is overlooked during counting because of aggregation of fibers.

TABLE 9

Fiber Length Distribution Summary

Number Medium Lengths in Micrometres

Preparation Technique	Chrysotile	Crocidolite			Taconite		
		Confirmed by SAED	Probable	Total Fibers	Confirmd by SAED	Probable	Total Fibers
Carbon Coated Nuclepore	0.72	0.87	0.43	0.66	1.2	0.92	1.1
Uncoated Nuclepore Jaffé Wick	0.75	0.88	0.70	0.70	1.2	1.0	1.1
0.45µm Millipore Jaffé Wick	0.68	0.90	0.63	0.63	1.6	0.90	1.3
0.45µm Millipore Condensation Washer	0.76	0.96	0.51	0.66	1.7	0.80	1.3
0.22µm Millipore Jaffé Wick	0.66	0.79	0.48	0.58	1.6	0.72	1.1
ORF Ashing Technique	0.80	0.95	0.63	0.79	1.2	0.75	1.1

TABLE 10

Summary of Principal Results

Item	Result at the 5% Significance Level	Significance Level Required to Reverse Decision
Is the Coated Nuclepore Preparation Mean Value significantly higher than any of the Millipore methods?	Yes (For all 3 materials)	<0.5
Are the losses using the 0.45μm Millipore and condensation washer significantly less than those using the 0.45μm Millipore and the Jaffé wick?	Chrysotile - Yes	1
	Crocidolite - No	20
	Taconite - No	30
Are the losses using the 0.22μm Millipore and the Jaffé wick significantly less than those using the 0.45μm Millipore and Jaffé wick?	Chrysotile - Yes	<0.5
	Crocidolite - Yes	0.5
	Taconite - Yes	<0.5
Are the losses using the 0.1μm Nuclepore and the Jaffé wick significantly higher than those using the Coated Nuclepore preparation?	Chrysotile - Yes	2.5
	Crocidolite - Yes	<0.5
	Taconite - No	*
Are the losses using the ORF ashing technique significantly higher than those using the direct Coated Nuclepore preparation?	Chrysotile - No	3.0
	Crocidolite - Yes	<0.5
	Taconite - No	*

* Result obtained significantly higher than that of Coated Nuclepore Technique

In many cases a group of fibers is so closely packed that none can be seen as fibers and are not included in the count. Evidently when using the ORF ashing technique, or the direct dissolution of Nuclepore filters, fibers are spread more uniformly on the grids and a greater percentage of them are available for inspection and checking by SAED. In a smaller way, this is an illustration of an effect which has been observed, particularly with chrysotile, where the ashing technique has reported a much higher value than did the carbon-coated Nuclepore technique. Particularly if large amounts of organic materials are present, fibers are observed to associate with the organic material and few of them can be identified either visually or by SAED. When the organic material is destroyed by ashing, the fibers become available for dispersion and inspection in the final preparation. Experience has shown that with organically dirty water containing slimes the direct carbon-coated Nuclepore technique can be somewhat misleading.

9.4 SUMMARY OF TECHNIQUE CHARACTERISTICS

Table 9 shows a summary of the fiber median lengths for the various preparation techniques. It can be seen that there is no significant shift in the median length of total fibers as a function of preparation technique.

Table 10 summarizes the principal results of this study. The significance of the reported loss is tested on each occasion at the 5% significance level, and a significance level is also reported which would be required to cause a reversal of the decision.

The carbon-coated Nuclepore technique gave significantly higher mean values than any of the Millipore techniques. No difference between the losses using the condensation washer, as opposed to the Jaffe washer, could be seen, except for a marginal one in the case of chrysotile. The ORF ashing technique did not introduce losses into preparations for chrysotile and taconite, although a 41% loss was demonstrated for crocidolite. The reason for this loss is not understood. A curious, but quite definite conclusion, is that the losses involved in the Jaffe washer preparation for all three fiber types are significantly less if a 0.22 μm pore size Millipore is used than if the 0.45 μm Millipore is used.

The Millipore direct dissolution methods were characterized by large fiber losses and large variabilities. Examination of the individual sample data shown in Appendix A shows that particularly in the case of chrysotile, use of the 0.45 μm Millipore with either the condensation washer or the Jaffe washer is capable of giving practically any value from zero up to those values yielded by the coated Nuclepore or ashing techniques. Therein lies, perhaps, some explanation for the controversy surrounding the use of the Millipore techniques. Even under these closely controlled operating conditions using clean fiber dispersions, the inevitable conclusion is that for chrysotile and crocidolite, which consisted largely or high aspect ratio fibers, the Millipore techniques are unreproducible. They are rather more consistent for the taconite. However, the mean losses for all three fiber types are high, between 60-80%, relative to the coated Nuclepore technique. A further indication of the extent of the losses when using these techniques

TABLE 11

Summary of Grid Uniformity and Fiber Loss Measurements

Technique	Chrysotile		Crocidolite		Taconite	
	Percentage of Samples with uniform Fiber distribution +	Fiber Loss % *	Percentage of Samples with uniform Fiber distribution +	Fiber Loss % *	Percentage of Samples with uniform Fiber distribution +	Fiber Loss % *
Carbon Coated 0.1µm Nuclepore	85	-	100	-	95	-
0.1µm Nuclepore Jaffe Wick	90	21	80	33	90	0
0.45µm Millipore Jaffe Wick	50	80	80	79	90	68
0.45µm Millipore Condensation Washer	25	59	70	71	70	64
0.22µm Millipore Jaffe Wick	10	57	60	52	90	50
ORF/Ashing	100	0	80	41	100	0

* Fiber Loss calculated assuming Coated Nuclepore Value as 100%

is given by the behavior of the chrysotile contamination in the Millipore filters. It is significant that this contamination was not detectable by any of the direct transfer preparation techniques; it only became apparent after an ashing procedure. Blanks processed simultaneously were satisfactory. A conclusion can be drawn that the direct transfer Millipore preparation techniques do not efficiently transfer material which is buried deeply within the filter structure. All of the 0.45 μm pore size Millipore filters used were contaminated to the same degree, and yet this was not noticeable using the direct transfer preparations. As discussed previously, it is logical to suggest that when using a 0.45 μm pore size Millipore filter, the deposited material penetrates more deeply into the structure than it does in the case of the 0.22 μm filter. The efficiency of deposition by the direct transfer technique appears to be related to its distance from the carbon film during the dissolution process. This conclusion is supported both by the fact that the fiber losses in the case of the 0.22 μm Millipore were less severe, and also by the behavior of the 0.45 μm Millipore chrysotile contamination. It is relevant to ask whether the direct transfer techniques would work even better using the 0.1 μm Millipore filter.

Table 11 shows a summary of the grid uniformity and fiber loss measurements for the six preparation techniques and the three types of asbestos fibers. It can be seen that chrysotile is the most difficult material to arrange in a uniform manner on the filter; in particular the 0.22 μm Millipore filter displayed only 10% of the samples which were uniform at a significance level of 0.1%, whereas 85% of the carbon-coated Nuclepore samples were uniform at that significance level. In the case of crocidolite and taconite the uniformity of all methods appears to be satisfactory. For example, using crocidolite with the 0.45 μm Millipore Jaffe washer preparation, 80% of the samples had uniform distributions on the grids. However, this is not to say that the preparation technique was satisfactory, since nearly 80% of the fibers had been lost. We are merely saying that those which remained were in fact relatively uniform in distribution. Thus in Table 11 uniformity data and the fiber loss data must be read together. The ORF ashing technique also has excellent uniformity of fiber distribution. This is to be expected, since after the ashing step the technique is identical with the carbon-coated Nuclepore method. The uncoated Nuclepore technique also yielded satisfactory grid uniformity, indicating minimal particle movement during preparation.

9.5 A DEMONSTRATION OF NEGLIGIBLE FIBER LOSS BY THE COATED NUCLEPORE TECHNIQUE

In the preceding analysis is has been assumed that the losses associated with the carbon-coated Nuclepore technique are effectively zero. Figures 27 to 32 demonstrate that this is indeed the case. Figures 27 and 28 are SEM and TEM micrographs respectively of a Nuclepore filter which has been used to filter a taconite tailings dispersion, shown at a magnification of 14,000. Figure 27, the SEM micrograph, was obtained after carbon coating the filter but before carrying out the washing operation in the Jaffe washer. Figure 28 shows a TEM micrograph of the same area of the filter replica after it had been washed in the Jaffe washer. It can be seen that all of the particles

The following illustrations are pairs of electron micrographs presented facing each other for comparison purposes.

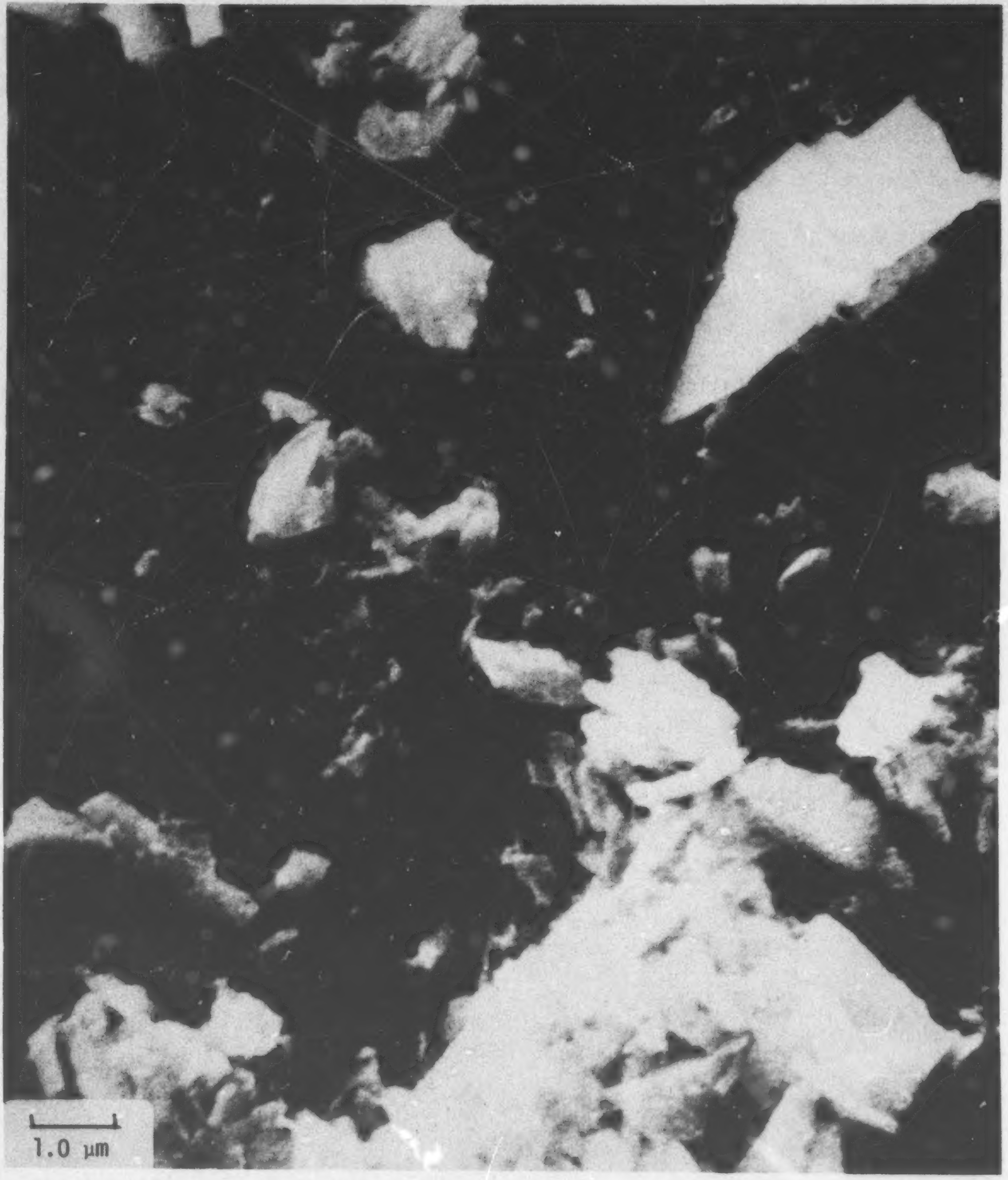


Fig. 27 SEM Micrograph of Carbon Coated Nuclepore Filter, Showing Taconite Deposit, Prior to Jaffe Washing
(Cambridge Stereoscan S4, 20 kV, carbon coating)

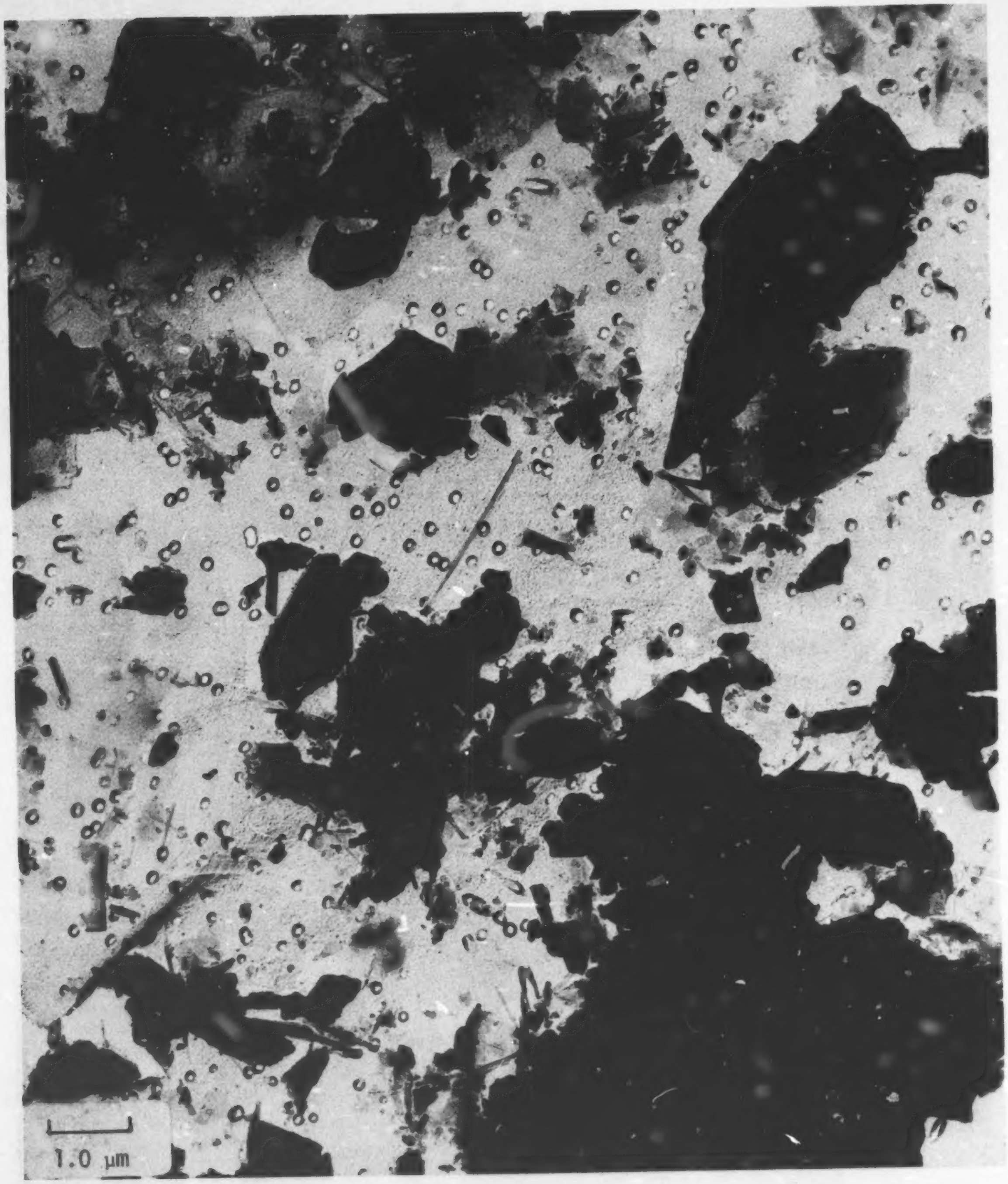


Fig. 28 TEM Micrograph of Carbon Replica After Jaffe Washing, Showing the Same Area of Deposit as in Fig. 27. Note the Correct Relative Positions of the Particles, Indicating no Particle Loss or Movement
(Philips EM301, 80 kV)

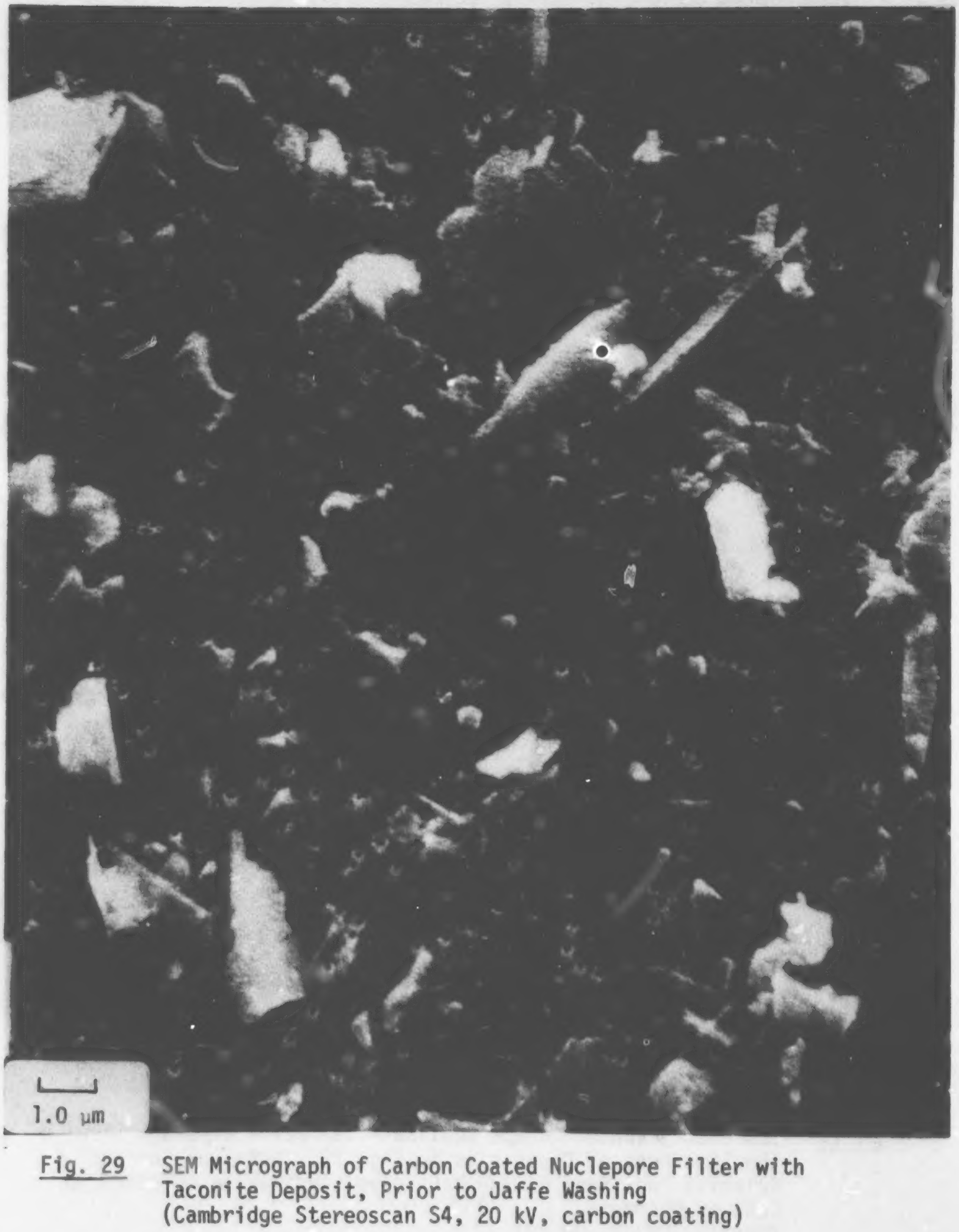


Fig. 29 SEM Micrograph of Carbon Coated Nuclepore Filter with Taconite Deposit, Prior to Jaffe Washing (Cambridge Stereoscan S4, 20 kV, carbon coating)

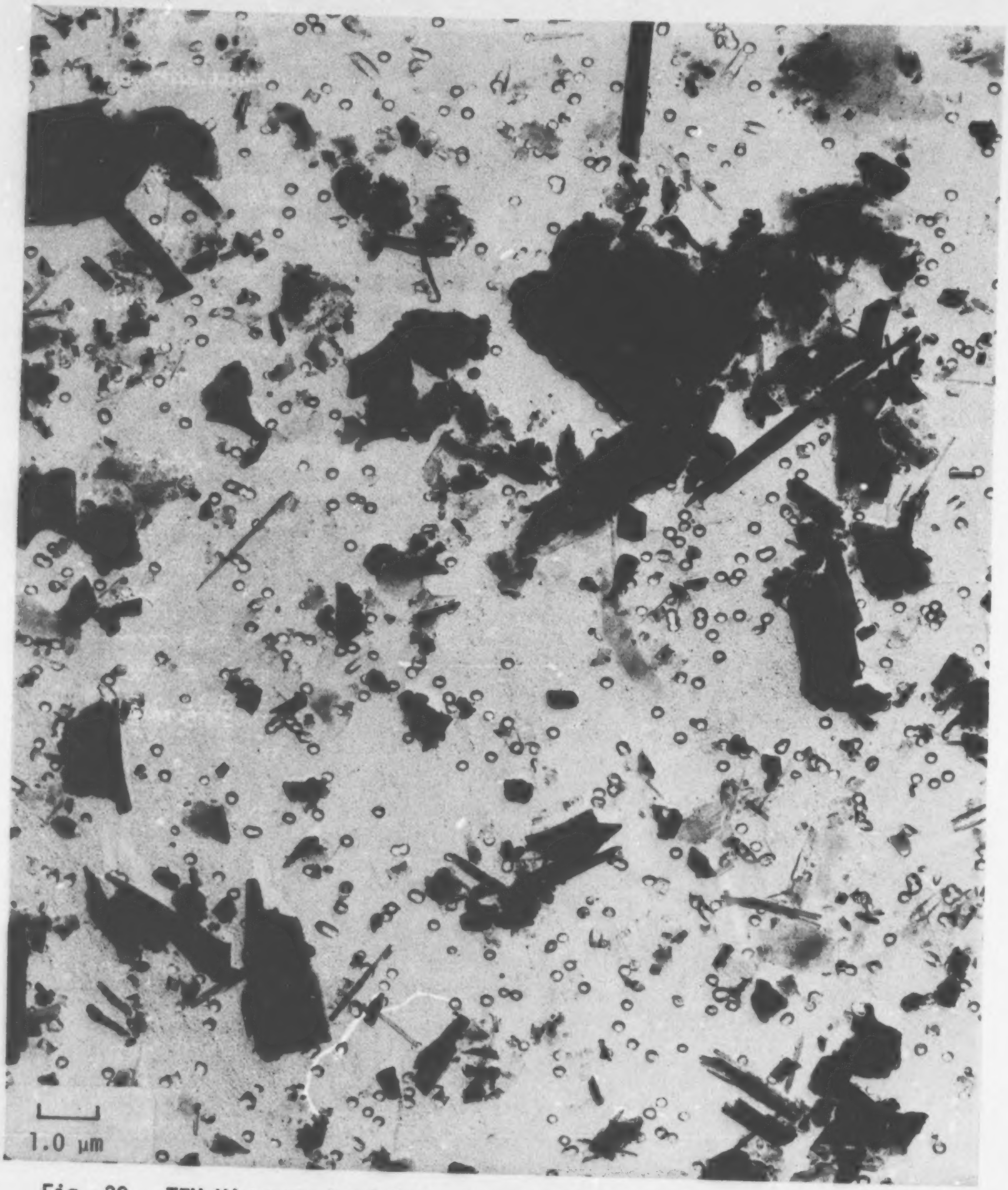


Fig. 30 TEM Micrograph of Carbon Replica After Jaffe Washing, Showing the Same Area of Deposit as in Fig. 29. Note that Even the Very Fine Fibers Visible in the TEM Image can be Traced on the SEM Image
(Philips EM301, 80 kV)

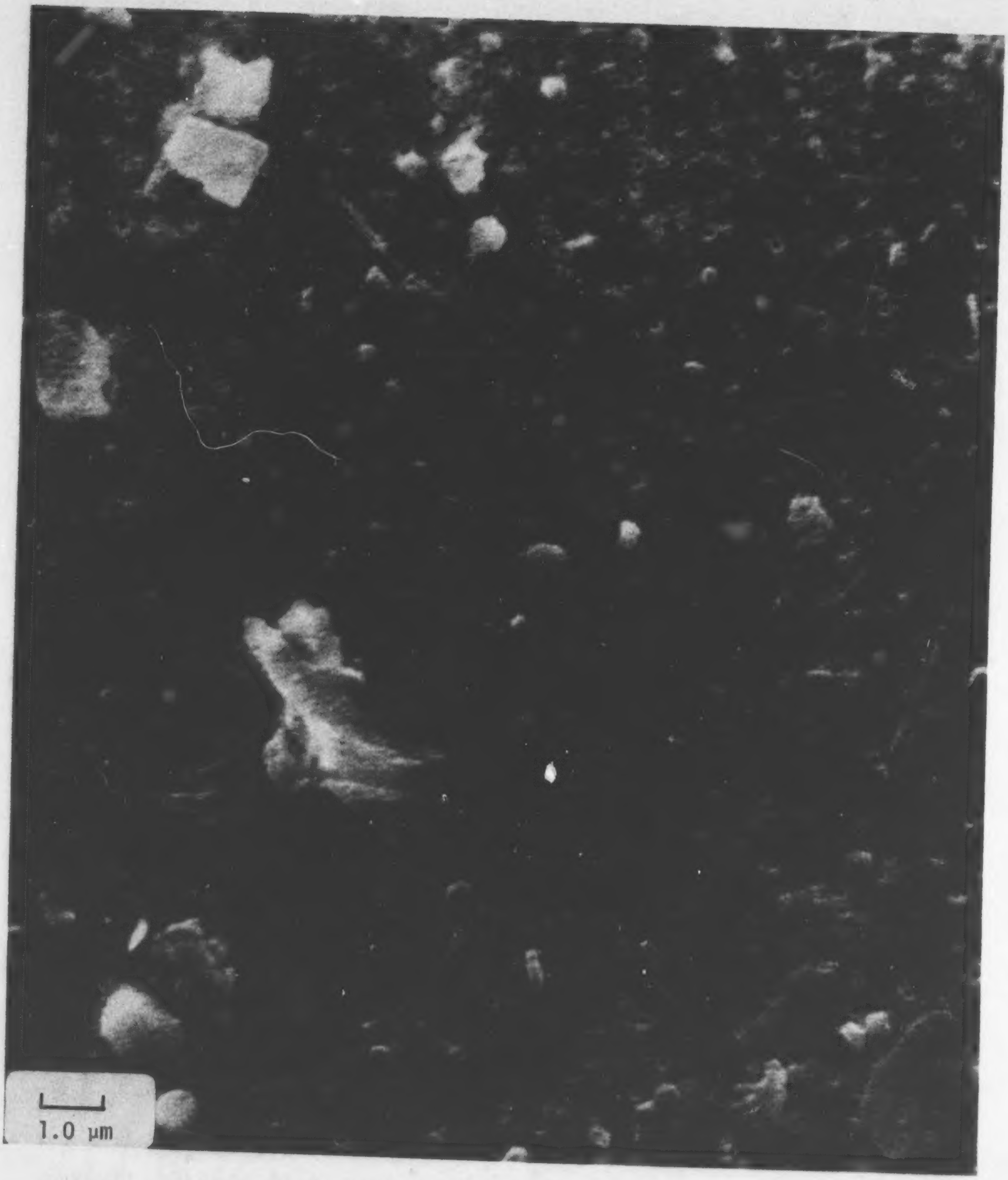


Fig. 31 SEM Micrograph of Carbon Coated Nuclepore Filter with Chrysotile Deposit, Prior to Jaffe Washing. Note Very Low Contrast of Chrysotile Fibrils
(Cambridge Stereoscan S4, 20 kV, carbon coating)

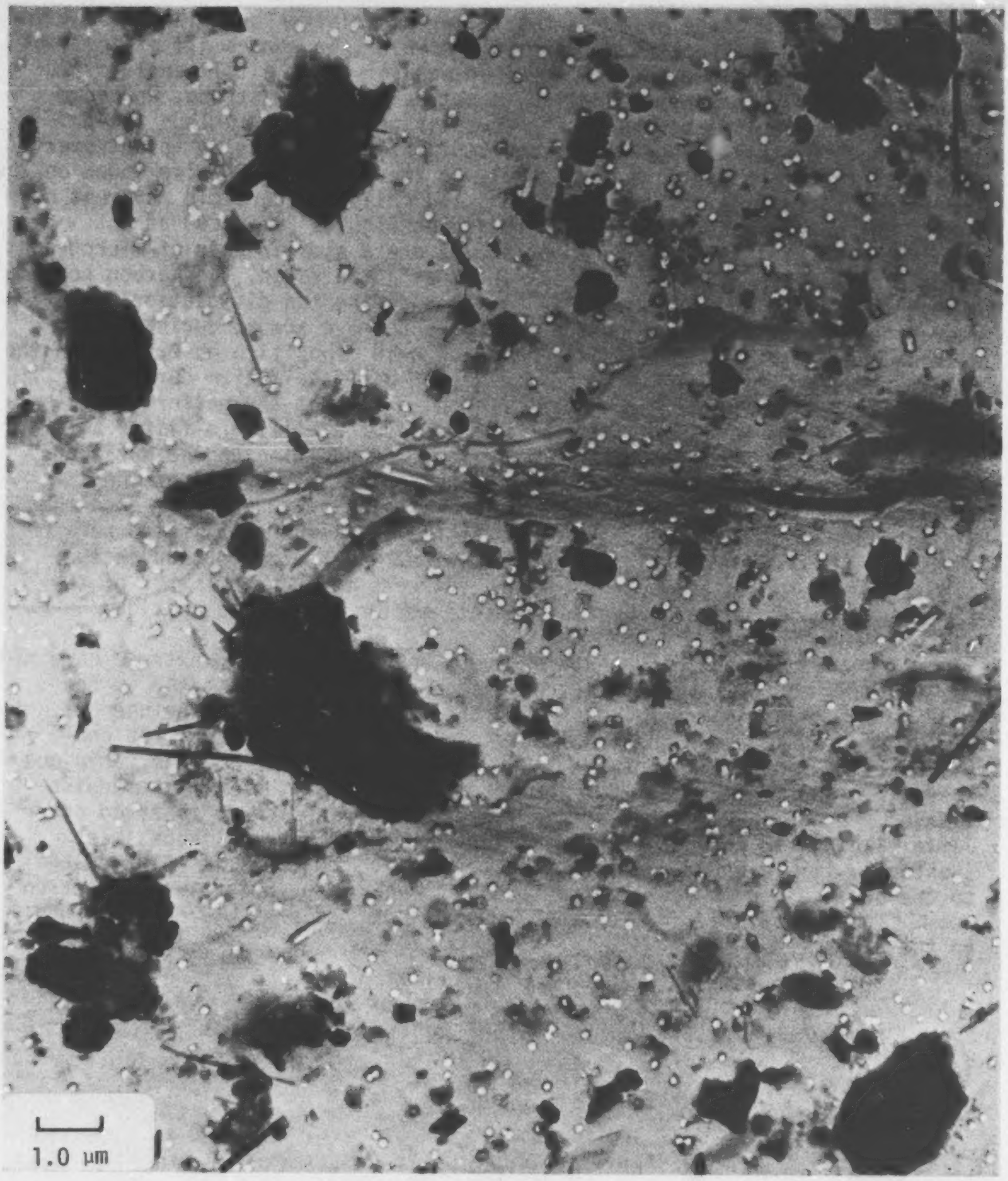


Fig. 32 TEM Micrograph of Carbon Replica After Jaffe Washing, Showing the Same Area of Deposit as in Fig. 31. Note that all Detectable Fibrils and Other Particles have been Retained in Position Indicating No Particle Loss
(Philips EM301, 80 kV)

present on the SEM image prior to the filter dissolution are also present in the TEM image of the replica. There will however, be some small distortions of the two images, since the SEM specimen was viewed at a tilt angle of 20 degrees; secondly the carbon replica is not particularly strong, and some sagging of the replica occurs during the washing operation. Thus the projected image of this is somewhat distorted. However, it is an incontrovertible fact that each particle discernible on the SEM image is also found on the TEM image in the same relative position, indicating no particle movement or loss. The same conclusion is drawn regarding Figures 29 and 30 at a magnification of 10,000. Figures 31 and 32 are a similar pair of micrographs using a chrysotile asbestos sample. Thus, as anticipated, the carbon coating embeds the fibers and holds them in position during the dissolution operation, whereas other techniques allow the particles to move around during the washing operation. The non-uniformities of grid deposition observable in the Millipore preparations are also indicative of particle motion during the washing operation, since there is no reason to suspect the uniformity of deposition on the Millipore filter. The use of the Millipore filter as a backing filter to achieve better uniformity of deposition on the Nuclepore filter indicates that the pressure distribution at the surface of the Millipore during the filtration operation is relatively uniform, hence the distribution of particles on the surface of the Millipore will also be uniform. Any differences in the uniformity of fiber distribution in the actual counting results would therefore indicate that one or other of the techniques concerned had incurred particle motion during preparation.

It should be mentioned that for the SEM-TEM comparison, extreme care had to be exercised in limiting electron dosage and heating deposited in the surface of the Nuclepore filter during the SEM examination. The use of normal beam currents in the SEM leads to plastic cross linking, and consequently to insolubility of the upper layers in chloroform. The technique was to use the minimum beam current possible, so that the image was noise-limited. Even under these conditions, insoluble plastic was observed after Jaffe washing, to an extent that examination in the TEM was impossible. Eventually it was found possible to obtain SEM micrographs in which dosage to the area concerned was limited to that given by one pass of the electron beam. This, however, required that the micrographed area was never viewed on the SEM screen.

SECTION 10

EFFECTS OF ULTRASONIFICATION

Before conducting these experiments it was necessary to calibrate the ultrasonic energy dissipation of the probe in use. The technique to accomplish this is relatively simple. The ultrasonic probe is operated at a known power setting with its tip immersed in a beaker containing a known volume of water, and the rate of temperature rise is measured. This rate of temperature rise is then compared with that produced by dissipation of a known power from an electrical heater immersed in the same volume of water. It is thus possible to calibrate the ultrasonic energy deposition in the known volume of water as a function of setting on the ultrasonic generator.

The effect of ultrasonic treatment at a frequency of 20 kHz was tested at two values of power dissipation, 0.05 watt/ml and 0.5 watt/ml, using dispersions of chrysotile and crocidolite. The chrysotile dispersion was a natural sample from a contaminated lake in Northern Ontario, and the crocidolite dispersion was artificially prepared using UICC material. Approximately 100 ml of the dispersion was taken in a 250 ml beaker and ultrasonic treatment was administered at a known power for a measured time interval. Two filters were then prepared by the carbon-coated Nuclepore technique from the treated dispersion, and the grids prepared from these two filters were counted by two individual operators, A and B. The results obtained are shown in Tables A-24 - A-27. In Table A-27 for the 15 second exposure time, operator A reported that the grids obtained were very badly dispersed. A second pair of grids were prepared from the original filter, but no significant improvement was obtained. Thus this appears to be an example of a Nuclepore filtration problem of some kind.

Tables 12 and 13 show the results computed in terms of fibers/g. These values are obtained by dividing the reported mean fiber concentration by the mass concentration. This normalizes the mean fiber concentration to a constant mass, and compensates to some extent for the inevitable sampling errors. It can be seen in Tables 12 and 13 that the only apparent increase in fiber numbers per gram occurs in the case of chrysotile at the high power and at 300 seconds exposure, although even here there is a discrepancy between the results obtained by operator A and operator B. No consistent effect can be seen in the case of crocidolite. Figures 33 and 34 show the changes in fiber number concentrations, plotted graphically, for operators A and B, together with the 95% confidence intervals. Although a consistent increase can be seen for chrysotile, no such increase can be seen in the case of crocidolite. Figures 35-42 show size distributions for the dispersions as functions of power and exposure time to the ultrasonic treatment, in which no consistent shift in the size distributions can be seen as a function of

TABLE 12

Effects of Ultrasonification

Aqueous Chrysotile Dispersion, Fibers/g x 10^{13}

Exposure Time, Seconds	Power = 0.05 watt/ml		Power = 0.5 watt/ml	
	Operator A	Operator B	Operator A	Operator B
0	2.15	2.29	2.22	2.08
15	1.38	1.94	1.42	1.39
60	1.43	1.87	1.22	1.68
300	1.62	2.09	2.17	2.92

TABLE 13

Effects of Ultrasonification

Aqueous Crocidolite Dispersion, Fibers/g x 10^{13}

Exposure Time, Seconds	Power = 0.05 watt/ml		Power = 0.5 watt/ml	
	Operator A	Operator B	Operator A	Operator B
0	0.76	0.69	0.70	0.81
15	0.71	0.62	* 0.98	0.83
60	0.65	0.80	1.04	0.83
300	0.59	0.79	0.62	0.60

* Poor grid distribution reported by operator

Figure 33 **Effects of Ultrasonification, Chrysotile**

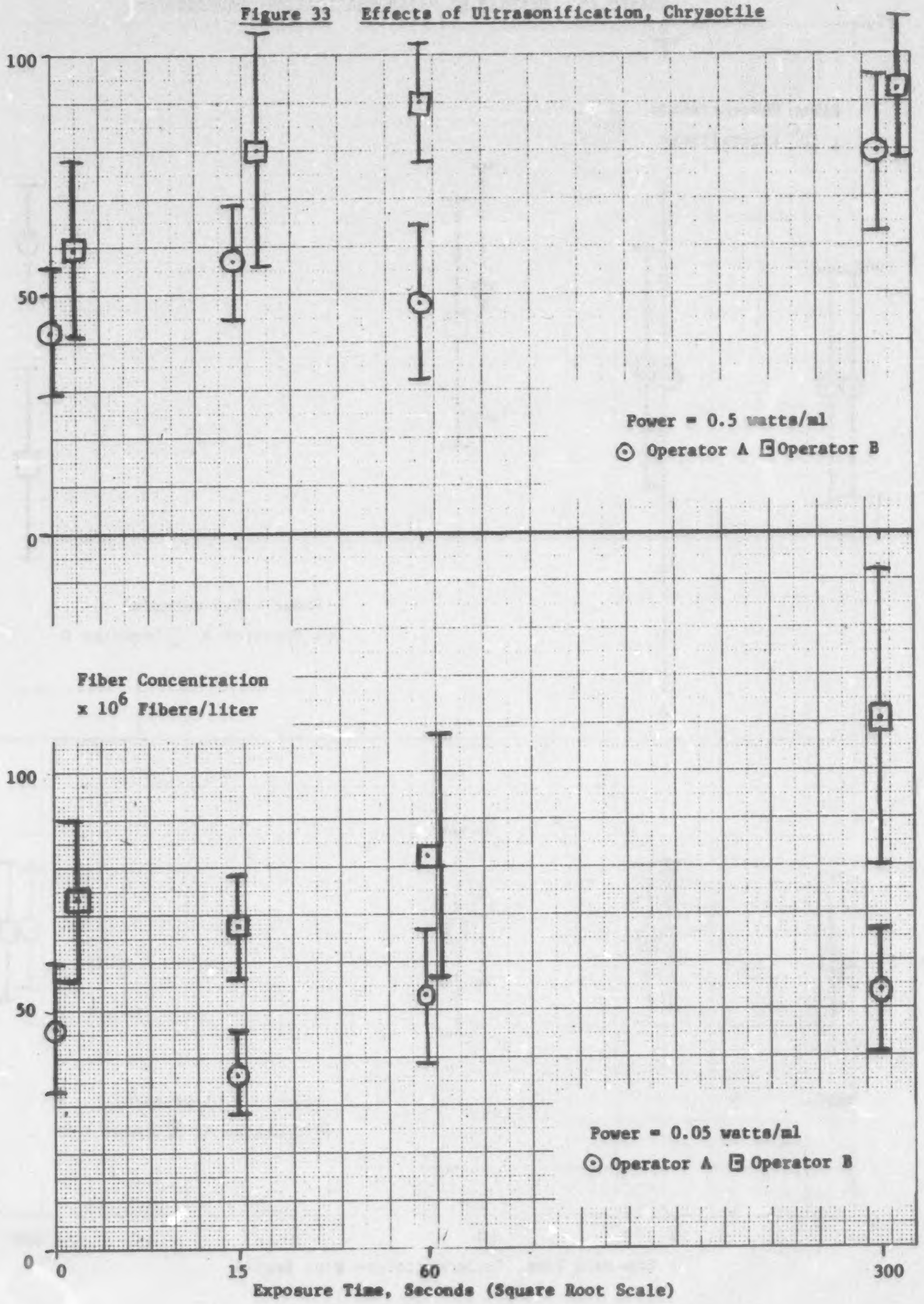


Figure 34 Effects of Ultrasonification, Crocidolite

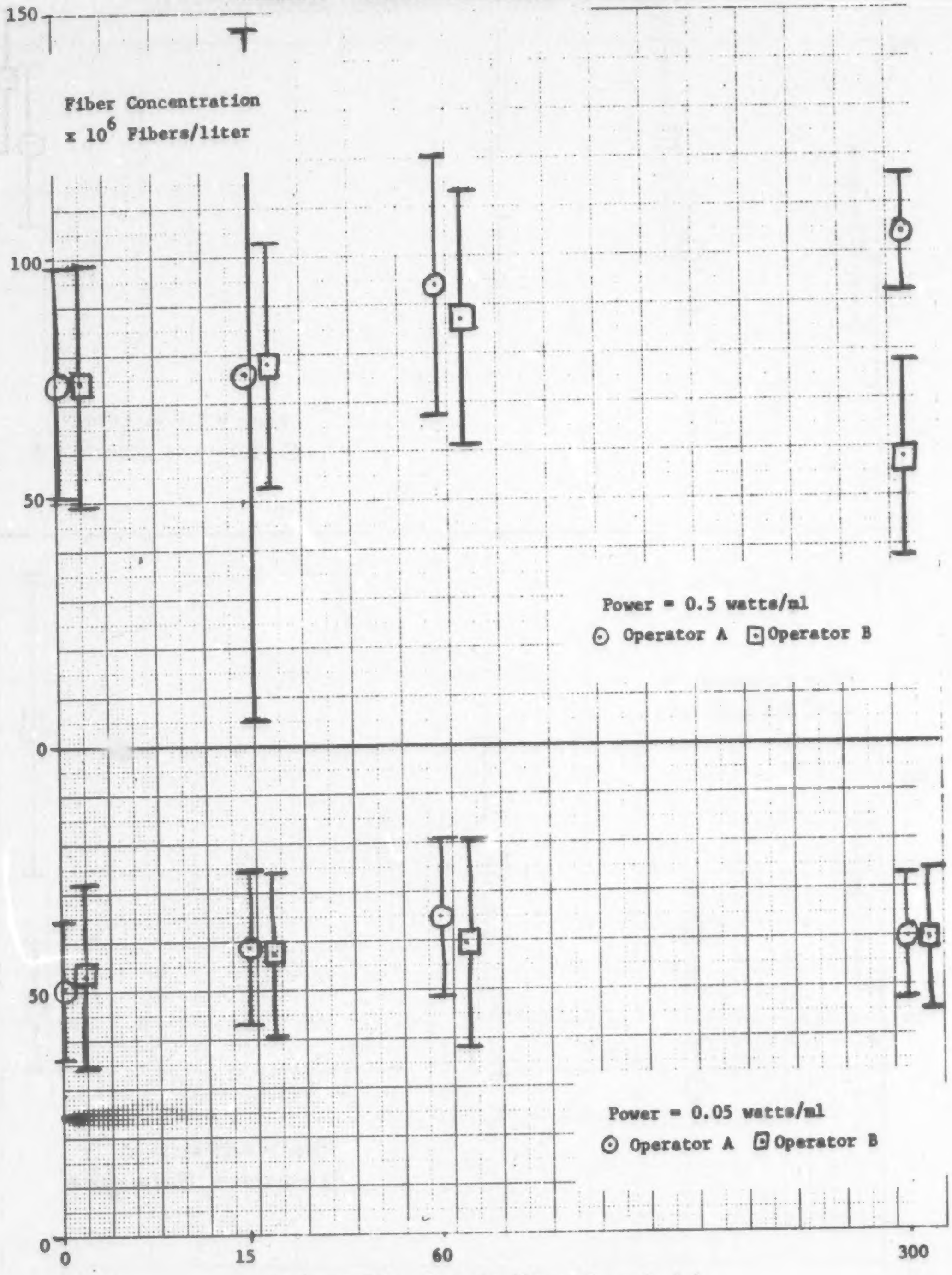


Figure 35 **Effects of Ultrasonification, Chrysotile Size Distributions**

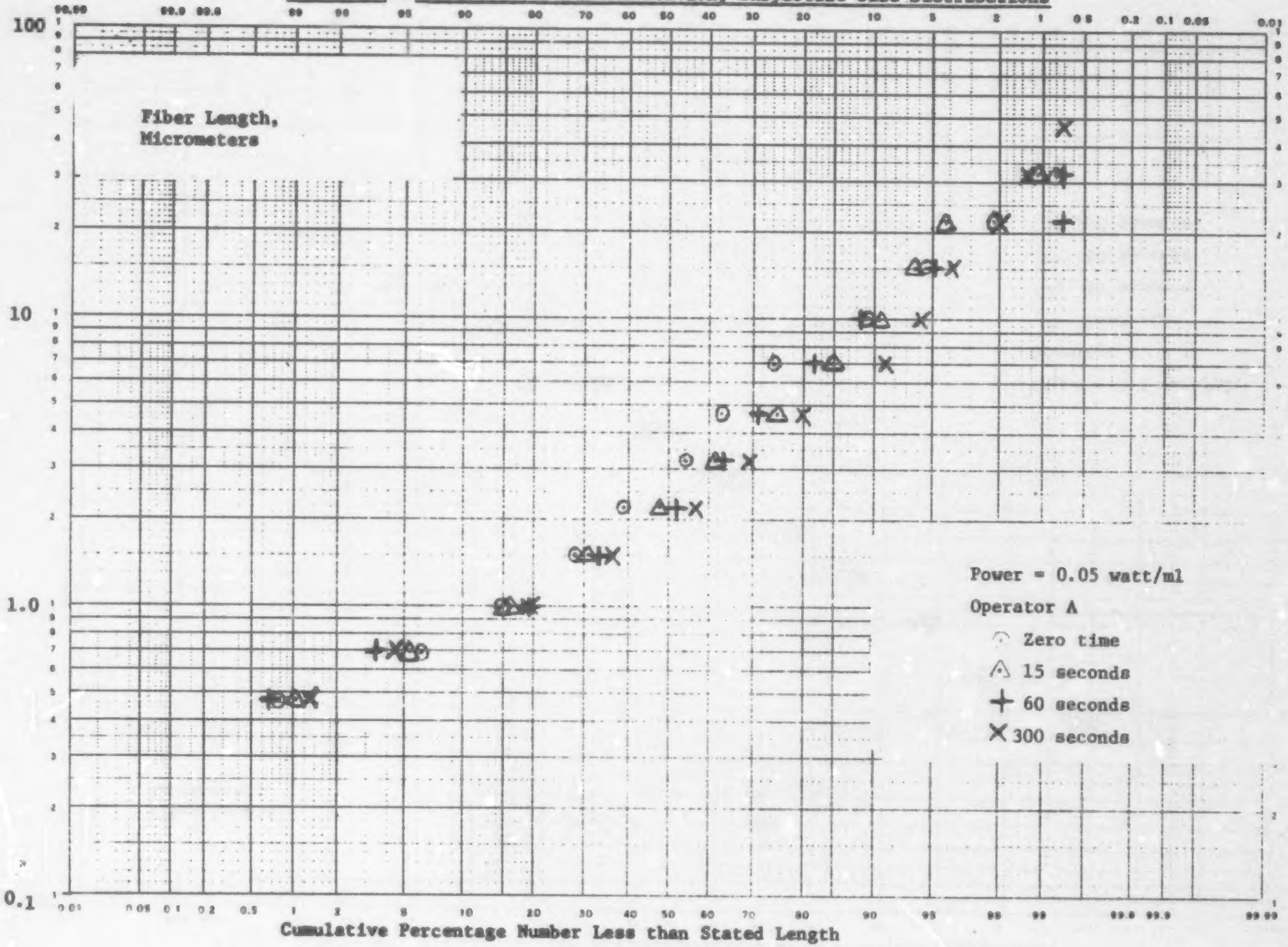


Figure 36 Effects of Ultrasonification, Chrysotile Size Distributions

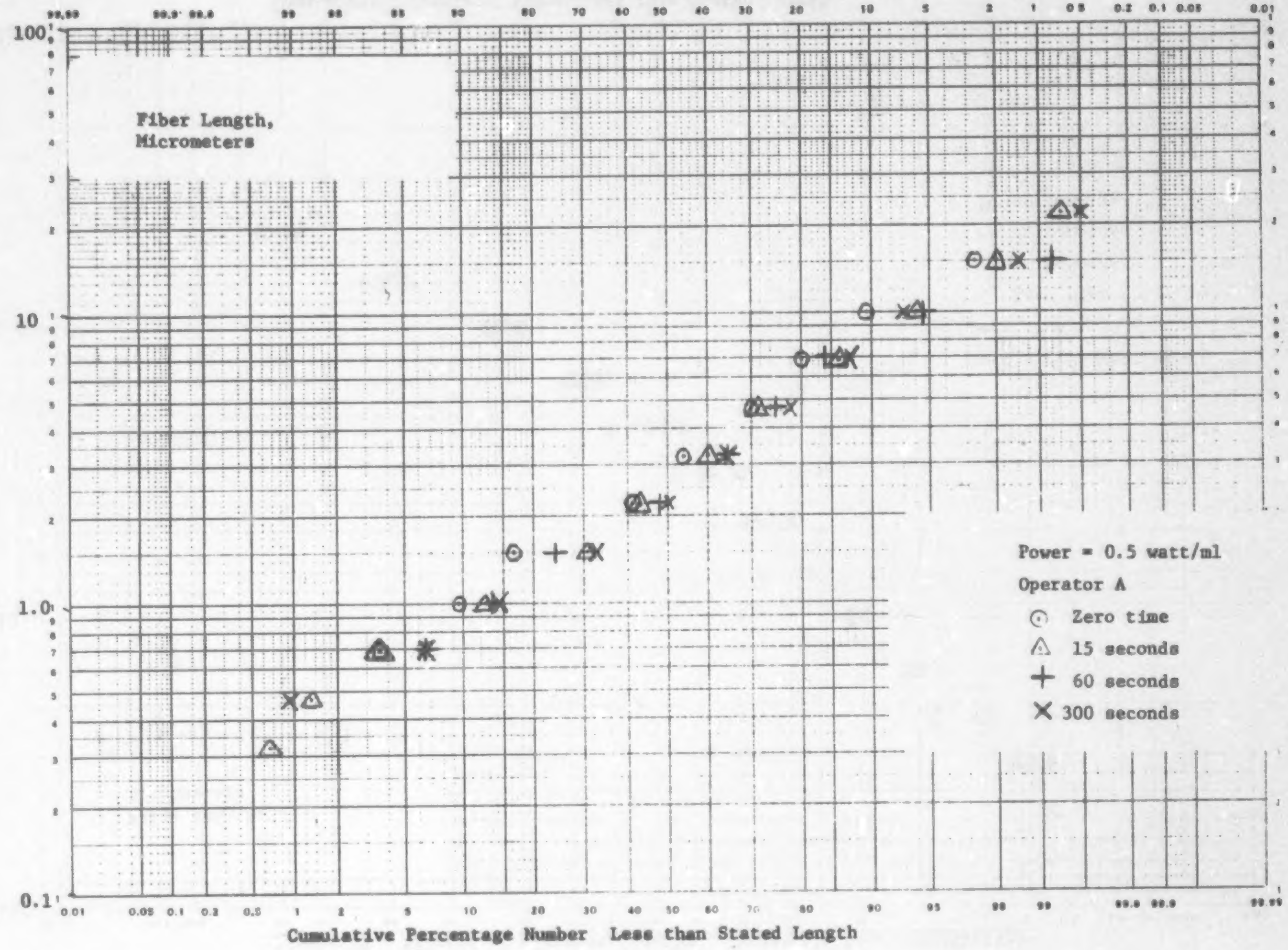


Figure 37 Effects of Ultrasonification, Chrysotile Size Distributions

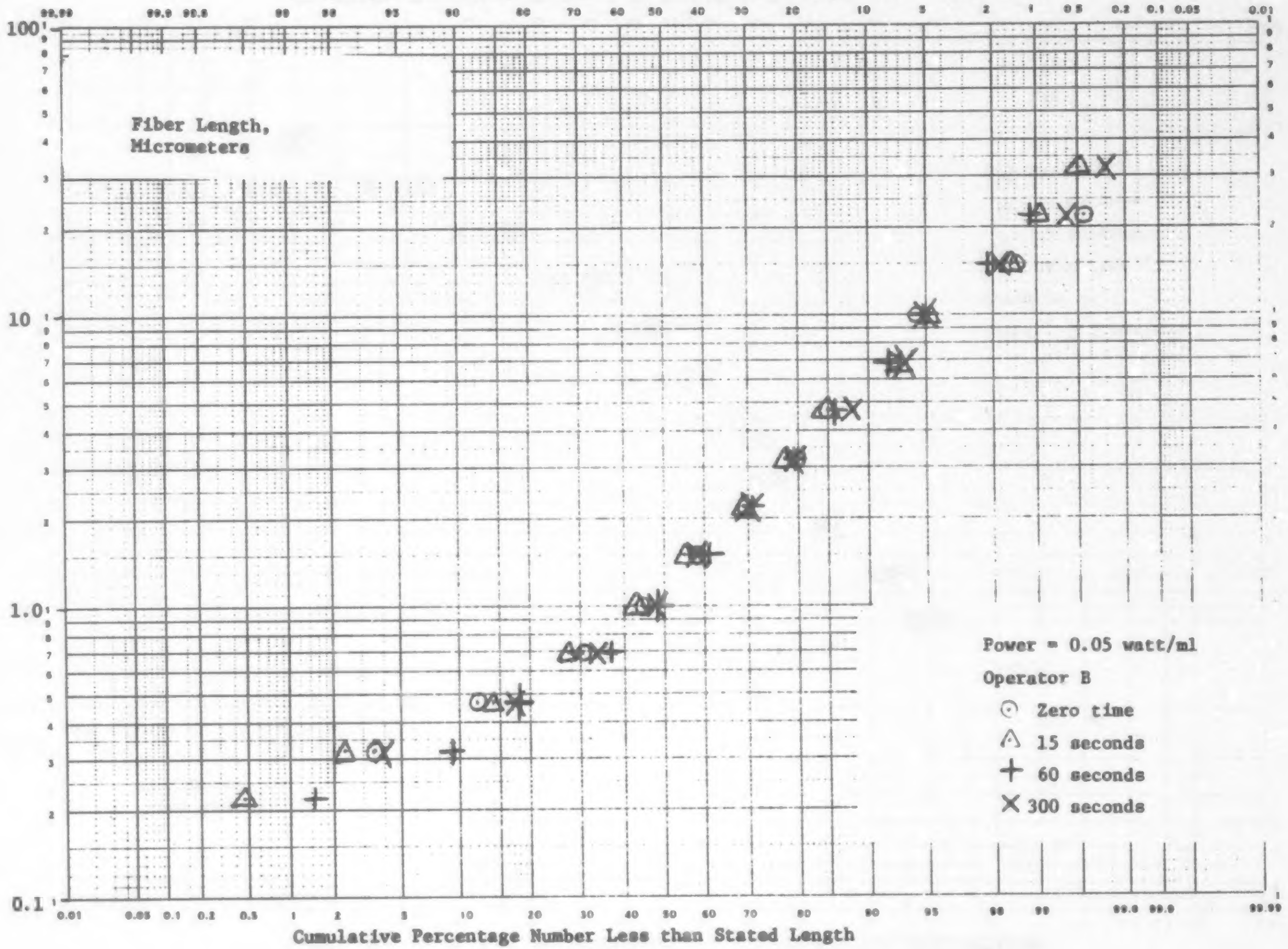


Figure 38 **Effects of Ultrasonification, Chrysotile Size Distributions**

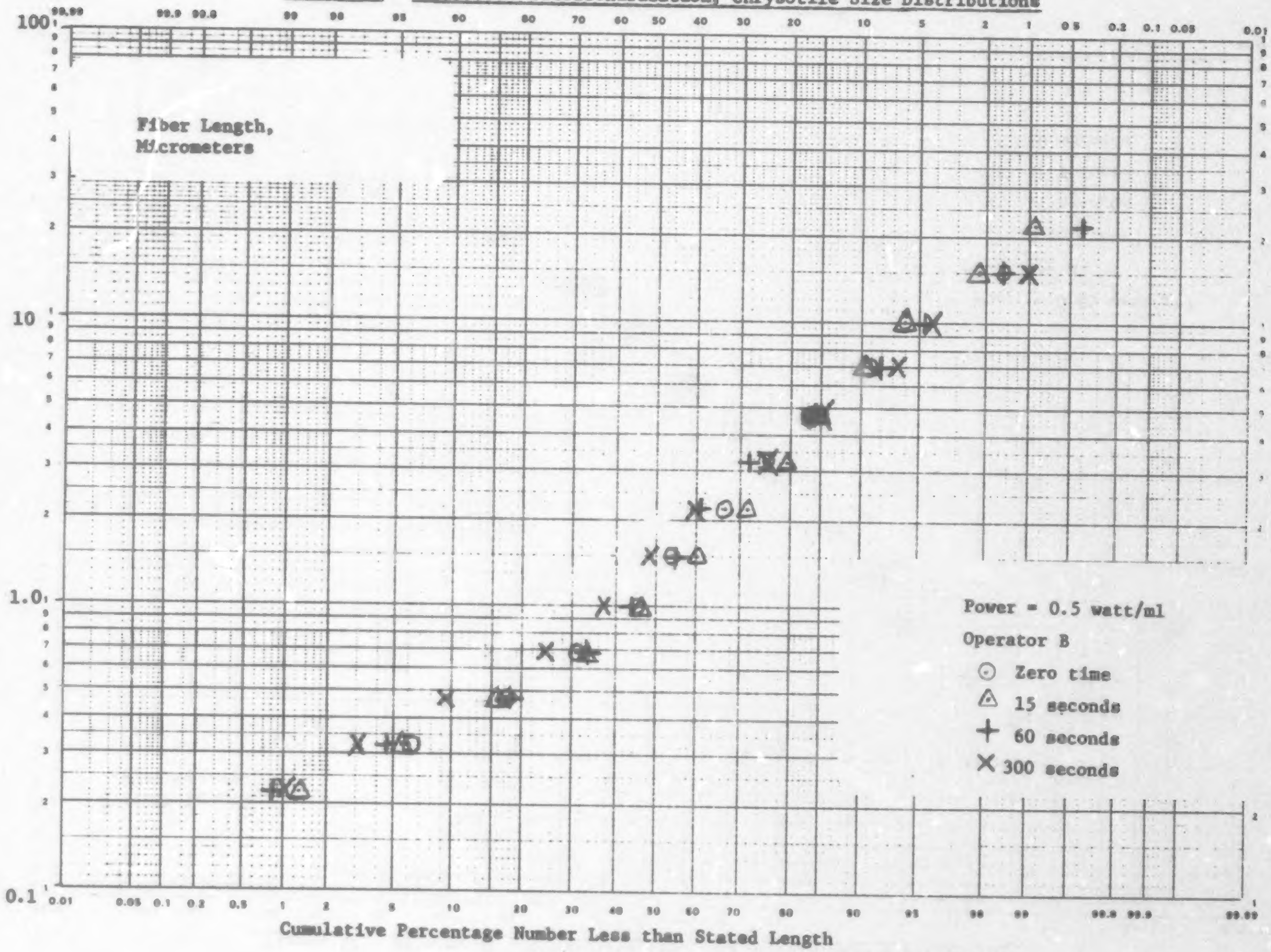


Figure 39 **Effects of Ultrasonification, Crocidolite Size Distributions**

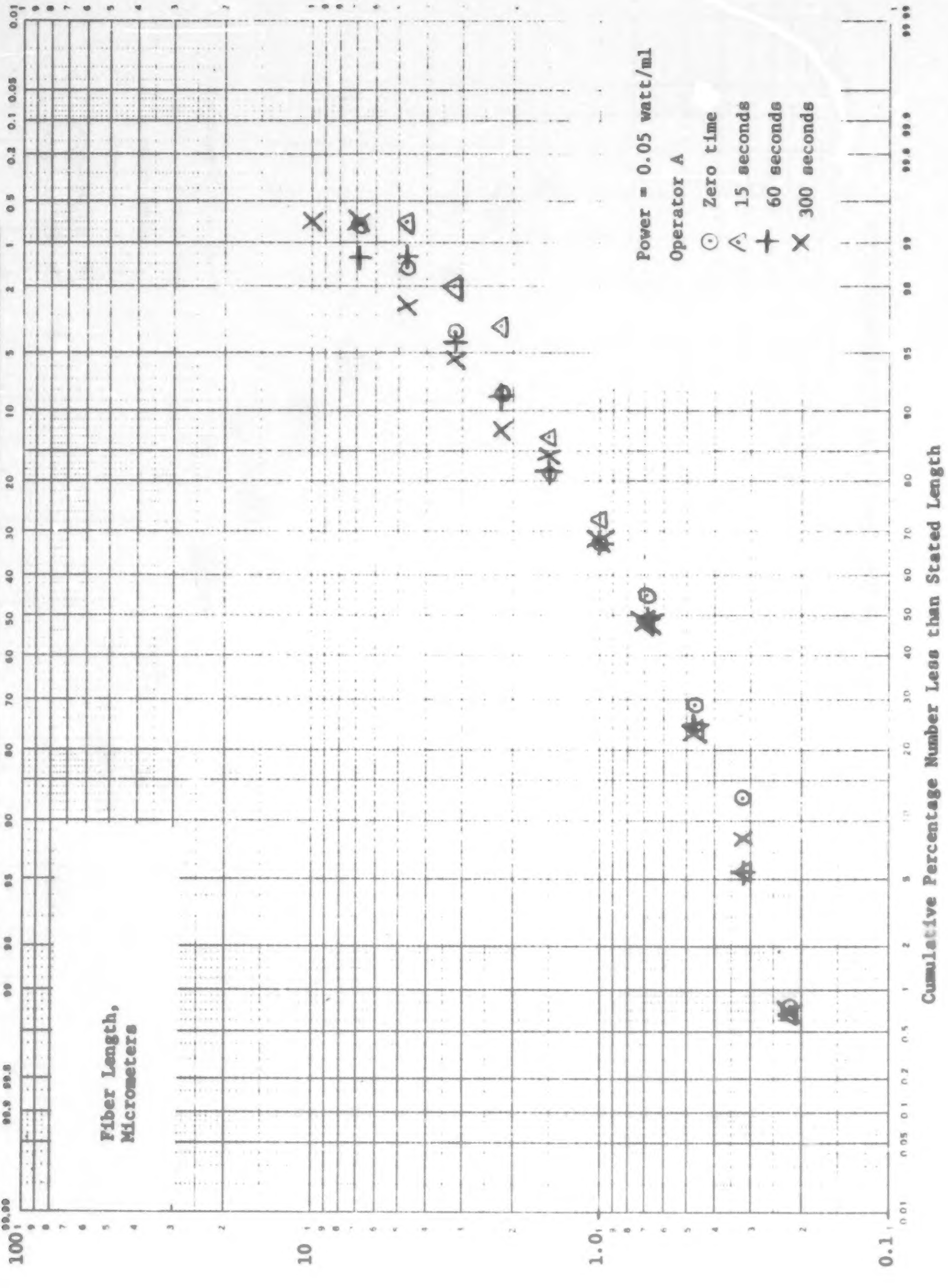


Figure 40 Effects of Ultrasonification, Crocidolite Size Distributions

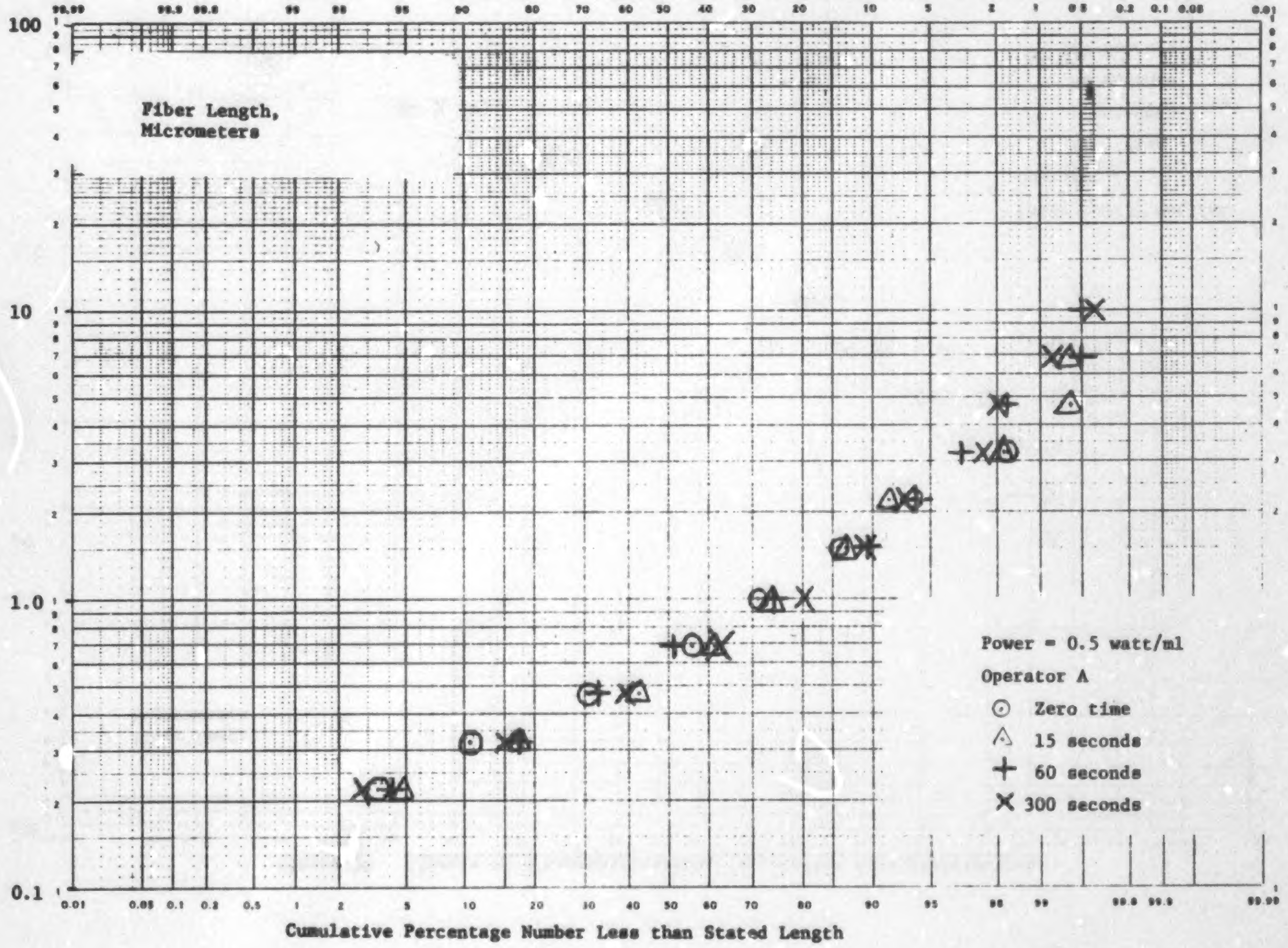


Figure 41 Effects of Ultrasonification, Crocidolite Size Distributions

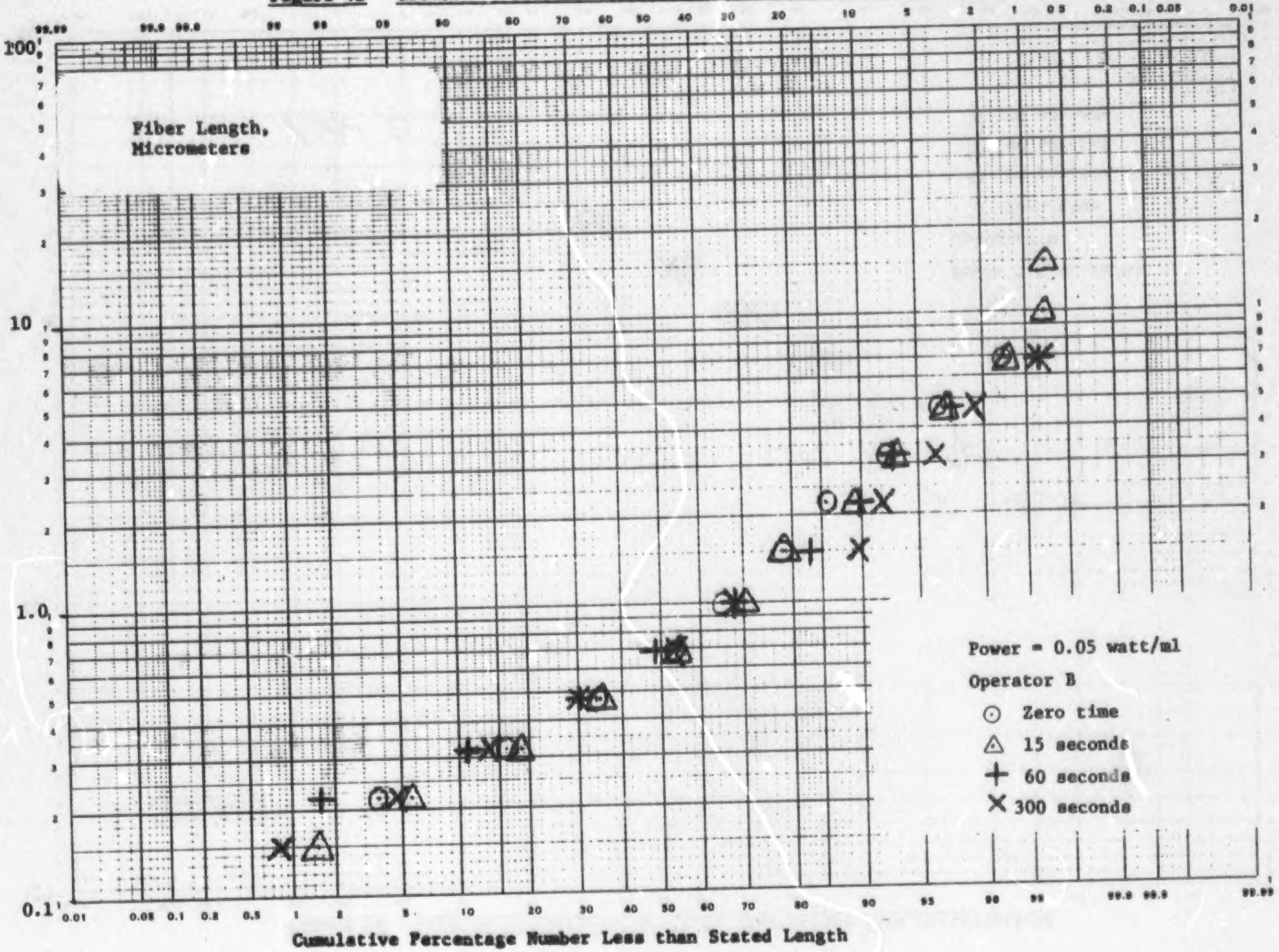
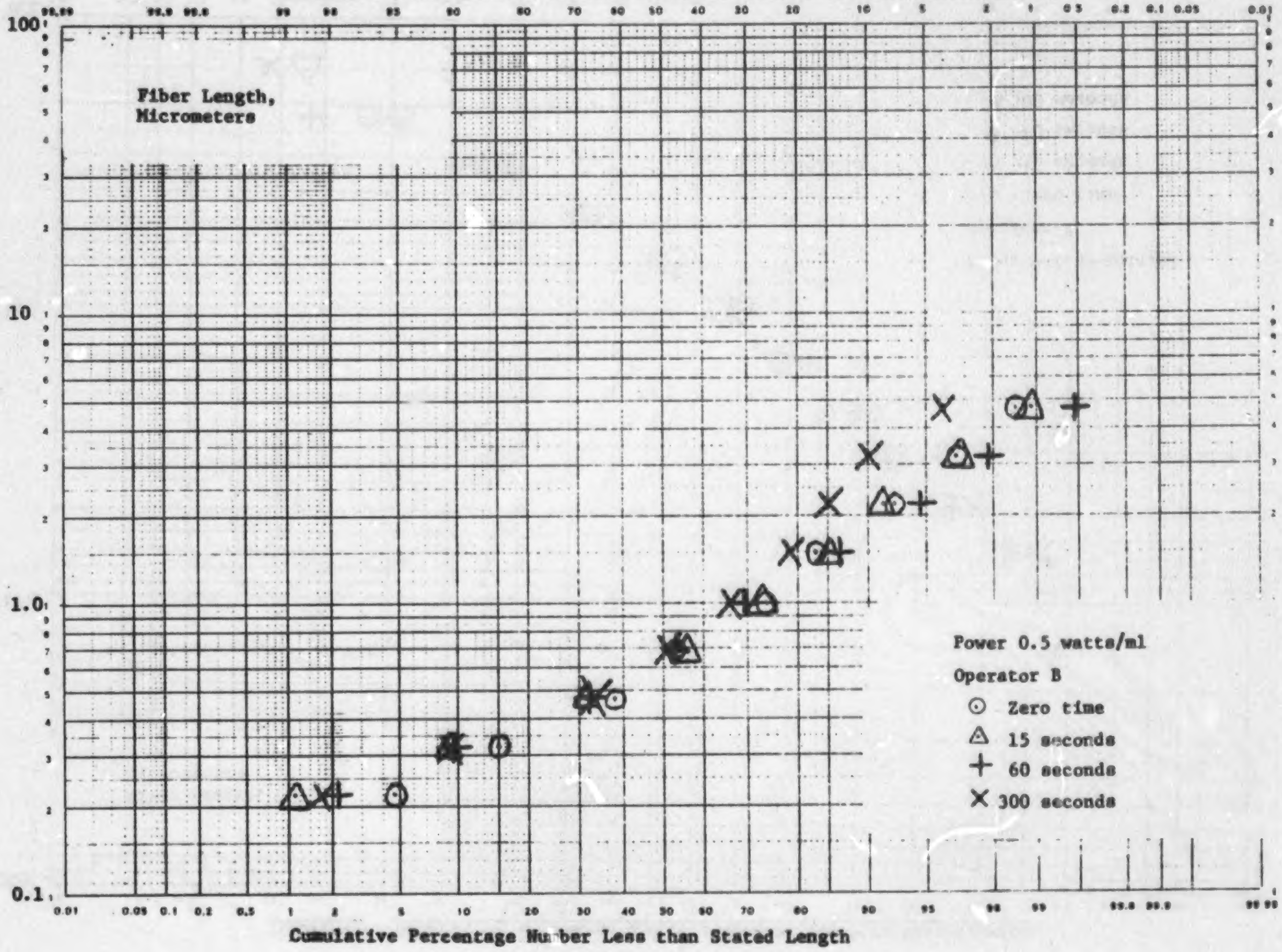


Figure 42 **Effects of Ultrasonification, Crocidolite Size Distributions**



exposure time or power. The summarized results of the effects of ultrasonification on the two dispersions are shown in Tables 14 and 15, in which a statistical analysis is carried out at 5% significance. In Table 14 for chrysotile, it can be seen that no significant effect was demonstrated at a power of 0.05 watt/ml up to times of 60 seconds. At the higher power, all periods of exposure demonstrated a statistically significant effect. However, even at the high power of 0.5 watt/ml for an exposure time of up to 60 seconds, the increase in fiber numbers was only 35%, which is at the limits of normal measurement accuracy for this kind of analysis. In Table 15 no statistically significant effect was demonstrated for fiber concentration change at 5% significance at any power or exposure time. In Tables 14 and 15 the observations of operators A and B are combined. Figures 43 and 44 show these results plotted graphically, and expressed as percentages of the initial value of fiber concentration. In Figure 43, it can be seen that if an approximately linear effect were to be assumed, the increase in fiber concentration as a function of time was approximately 0.1% per second at 0.05 watt/ml, whereas at 0.5 watt/ml the rate of increase was approximately 0.2% per second. Consideration of Figure 44 indicates graphically that no consistent effect on the fiber concentration was demonstrated as a function of either power or exposure time for crocidolite.

TABLE 14

Effects of Ultrasonification

Chrysotile: Comparison of Mean Grid Square Fiber Counts

(Combined Observations of Operators A and B)

Power Density watt/ml	Exposure Time Seconds	Mean Grid Square Fiber Count *	Sample Standard Deviation ✦	Increase over initial value %	Detectable Increase at 5% Significance	Approximate Significance Level Required for Change of Decision, %
0.05	0	17.61	7.29	-	-	-
	15	15.18	5.94	-13.8	No	20
	60	19.83	9.58	12.6	No	30
	300	24.29	12.72	37.9	Yes	2.5
0.50	0	14.68	7.07	-	-	-
	15	19.87	8.12	35.4	Yes	1
	60	19.69	7.96	34.1	Yes	1
	300	24.90	6.58	69.6	Yes	<0.5

* Normalized to mean grid opening area

✦ Total number of grid openings counted = 20

TABLE 16

Effects of Ultrasonification

Crocidolite: Comparison of Mean Grid Square Fiber Counts

(Combined Observations of Operators A and B)

Power Density watt/ml	Exposure Time Seconds	Mean Grid Square Fiber Count	Sample Standard Deviation✦	Increase over initial value %	Detectable Increase at 5% Significance	Approximate Significance Level Required for Change of Decision, %
0.05	0	12.28	5.75	-	-	-
	15	13.95	5.39	13.6	No	20
	60	14.89	5.91	21.3	No	10
	300	14.35	4.22	16.9	No	20
0.5	0	16.63	7.15	-	-	-
	15	17.31	16.11[(a)]	4.1	No	>40
	60	20.25	7.88	21.8	No	10
	300	18.00	7.24	8.2	No	30
0.5+	0	14.70	6.88	-	-	-
	15	17.70	7.89	20.4	No	40

* Normalized to mean grid opening area

✦ Total number of grid openings counted = 20

+ Results for Operator A only, 10 grid openings

(a) Very poor grid distribution, additional result for Operator A only is appended.

Figure 43 **Effects of Ultrasonification: Percentage Increase as a Function of Exposure Time - Chrysotile**

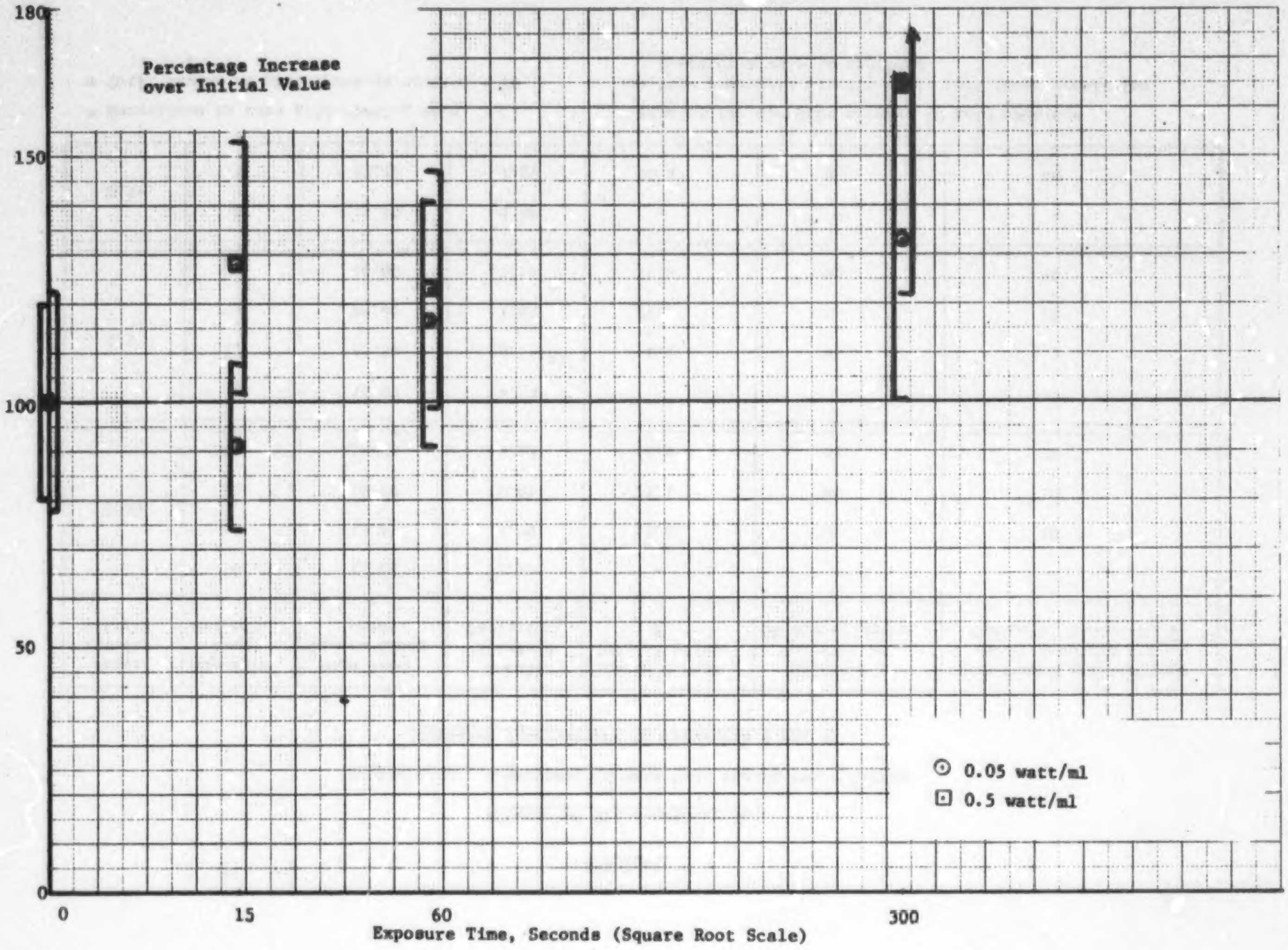
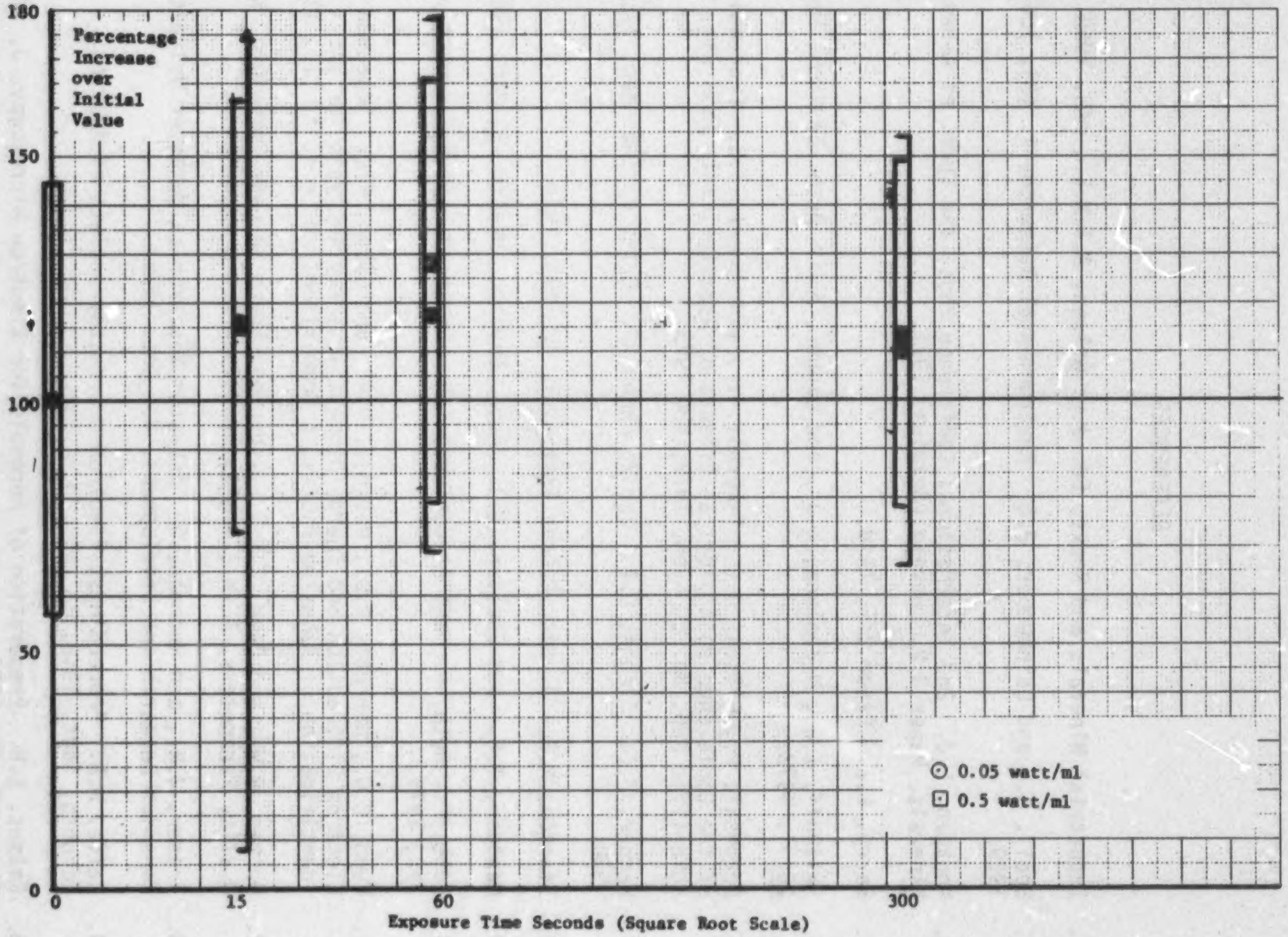


Figure 44 Effects of Ultrasonification: Percentage Increase as a Function of Exposure Time - Crocidolite



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APPENDIX A

INDIVIDUAL SAMPLE RESULTS

TABLE A-1

Carbon Coated Nuclepore Technique (Initial 10 Samples)

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration µg/1	Grid Distribution	
				χ^2	Significance Level of Uniformity %
5	36.9	28.6 - 45.2	0.284	19.47	1
10	46.4	33.1 - 59.7	0.214	43.09	<0.1
15	32.1	26.0 - 38.3	0.185	14.84	5
20	33.2	24.5 - 41.9	0.201	27.03	0.1
25	33.3	24.2 - 42.4	0.667	28.25	<0.1
30	20.5	14.5 - 26.5	0.127	20.28	1
35	18.0	9.59 - 26.4	0.110	192.14	<0.1
40	17.0	10.7 - 23.3	0.136	26.38	0.1
45	14.5	8.16 - 20.8	0.144	34.21	<0.1
50	16.5	11.0 - 22.0	0.140	20.49	1
5 * +	30.9	20.1 - 41.7	0.360	24.22	0.1
10 * ✦	16.2	8.90 - 23.5	0.953	20.24	0.5
15 *	25.7	14.7 - 36.7	0.899	11.23	2.5
30 *	21.6	12.5 - 30.7	0.402	25.58	0.1
35 *	10.6	6.00 - 15.2	0.475	26.59	0.1
40 *	10.7	7.40 - 14.0	0.418	9.22	10

* Repeat Preparations

+ 9 grid square count

✦ 6 grid square count

TABLE A-2

Carbon Coated Nuclepore Technique (Second 10 Samples)

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration µg/l	Grid Distribution	
				χ^2	Significance Level of Uniformity %
3	30.0	23.0 - 37.0	0.466	12.34	10
7	24.1	19.1 - 29.1	0.298	11.04	10
13	25.6	19.0 - 32.2	0.286	18.33	2.5
17	24.1	18.3 - 29.9	0.314	18.86	2.5
23	27.4	19.5 - 35.3	0.418	22.54	0.5
27	21.1	16.4 - 25.8	0.166	15.19	5
33	22.8	17.3 - 28.3	0.187	10.61	10
37	15.6	11.7 - 19.5	0.080	7.85	10
43	17.3	14.2 - 20.4	0.164	3.67	10
47	16.9	12.6 - 21.2	0.225	12.16	10

TABLE A-3

Jaffe Wick Technique, 0.1μm Nuclepore

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/l	Grid Distribution	
				χ^2	Significance Level of Uniformity %
5	34.6	28.1 - 41.1	0.443	21.23	1
10	23.7	17.1 - 30.3	0.247	16.39	5
15	25.2	14.5 - 35.9	0.232	44.82	<0.1
20	21.0	16.3 - 25.7	0.199	12.43	10
25	19.7	15.4 - 24.0	0.239	12.33	10
30	20.3	14.1 - 26.5	0.151	19.40	1
35	13.9	8.70 - 19.1	0.216	23.67	0.1
40	17.1	12.1 - 22.1	0.133	9.74	10
45	9.70	7.05 - 12.4	0.135	8.06	10
50	8.16	4.10 - 12.2	0.620	21.31	1
30 *	12.1	8.90 - 15.3	0.198	6.21	10
50 *	16.8	11.5 - 22.1	0.144	17.77	2.5

* Repeat Preparations

TABLE A-4

Jaffe Wick Technique, 0.45μm Millipore

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/l	Grid Distribution	
				χ^2	Significance Level of Uniformity %
5	6.18	0.88 - 11.5	0.165	52.03	<0.1
10	5.29	0 - 13.8	0.047	188.13	<0.1
15	10.2	7.60 - 12.8	0.133	12.20	10
20	7.80	0 - 18.1	0.153	137.37	<0.1
25	13.3	3.60 - 23.0	0.122	77.26	<0.1
30	2.38	0 - 5.65	0.072	64.15	<0.1
35	0.79	0 - 1.88	0.006	26.47	0.1
40	2.33	0 - 6.07	0.007	60.02	<0.1
45	5.12	1.86 - 8.38	0.040	21.73	1
50	2.64	0 - 5.40	0.032	37.53	<0.1
30 *	2.41	0.55 - 4.27	0.022	17.58	2.5
35 *	0.35	0 - 0.85	0.002	8.56	10
40 *	3.29	0.48 - 6.10	0.035	22.82	0.5
50 *	3.79	0.87 - 6.71	0.048	15.96	5

* Repeat Preparations

TABLE A-5

Condensation Washer Technique, 0.45μm Millipore

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/1	Grid Distribution	
				χ^2	Significance Level of Uniformity %
5	8.05	2.29 - 13.8	0.084	49.65	<0.1
10	10.6	0 - 22.1	0.089	132.41	<0.1
15	21.6	11.1 - 32.1	0.273	53.74	<0.1
20	8.80	3.53 - 14.1	0.099	26.79	0.1
25	17.9	10.7 - 25.1	0.159	32.61	<0.1
30	0.50	0 - 1.29	0.009	14.10	10
35	10.0	4.50 - 15.5	0.140	37.57	<0.1
40	12.7	5.10 - 20.3	0.105	35.45	<0.1
45	5.71	0.41 - 11.0	0.183	45.69	<0.1
50	4.85	0.22 - 9.48	0.041	35.00	<0.1
30 *	5.01	2.23 - 7.79	0.080	14.32	10
50 *	9.69	0 - 19.6	0.099	72.26	<0.1

* Repeat Preparations

TABLE A-6

Jaffé Wick Technique, 0.22μm Millipore

Chrysotile

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/l	Grid Distribution	
				χ^2	Significance Level of Uniformity %
2	12.5	3.30 - 21.7	0.081	73.21	<0.1
4	5.26	0.38 - 10.1	0.125	41.68	<0.1
6	16.1	7.00 - 25.2	0.131	65.55	<0.1
8	8.87	0.42 - 17.3	0.123	115.63	<0.1
10	9.02	2.72 - 25.3	0.074	48.07	<0.1
12	10.1	3.10 - 17.1	0.065	65.16	<0.1
14	4.99	1.47 - 8.51	0.102	29.35	<0.1
16	13.9	9.30 - 18.5	0.124	18.23	2.5
18	8.88	3.41 - 14.4	0.475	45.97	<0.1
20	9.93	3.23 - 16.6	0.399	60.44	<0.1
4 *	10.7	2.80 - 18.6	0.142	39.57	<0.1

* Repeat Preparation

TABLE A-7

ORF Ashing Technique: Chrysotile *

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/1	Grid Distribution	
				χ^2	Significance Level of Uniformity %
3	11.28	4.41 - 18.15	0.145	16.04	5
7	23.68	16.58 - 30.78	0.306	10.20	10
13	16.48	9.07 - 23.89	0.274	13.35	10
17	38.18	31.17 - 45.19	0.370	5.98	10
23	23.18	15.69 - 30.67	0.213	12.97	10
27	17.78	10.74 - 24.82	0.130	9.94	10
33	33.28	26.46 - 40.10	0.236	6.73	10
37	28.98	22.33 - 35.63	0.245	5.60	10
43	29.48	20.68 - 38.28	0.218	11.06	10
47	30.48	23.09 - 37.87	0.200	10.93	10

* Results corrected for contamination detected in Millipore polymer

TABLE A-8

Carbon Coated Nuclepore Technique (Initial 10 samples)

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	Mass Conc. μg/l	χ^2 (Total)	Significance Level of Uniformity %
5	14.4	9.8 - 19.0	1.88	6.43	3.68 - 9.18	0.08	20.8	1.96	26.73	0.1
10	7.38	4.71 - 10.1	2.13	7.38	3.66 - 11.1	0.21	14.8	2.34	15.32	5
15	12.7	8.10 - 17.3	1.35	6.37	3.73 - 9.01	0.10	19.1	1.45	6.08	10
20	6.84	3.80 - 9.88	1.75	7.26	3.99 - 10.5	0.23	14.1	1.98	18.93	2.5
25	8.72	3.32 - 14.1	2.53	7.06	3.90 - 10.2	0.19	15.8	2.72	23.04	0.5
30	8.72	6.96 - 10.5	2.07	4.46	2.27 - 6.65	0.07	13.2	2.14	5.50	10
35	8.23	5.79 - 10.7	1.11	3.82	2.22 - 5.42	0.03	12.1	1.14	7.46	10
40	11.0	7.40 - 14.6	2.31	4.33	2.89 - 5.77	0.05	15.3	2.36	8.59	10
45	11.0	8.00 - 14.0-	1.88	3.68	1.41 - 5.95	0.05	14.7	1.93	8.95	10
50	10.9	6.90 - 14.9	1.74	5.24	2.80 - 7.68	0.09	16.1	1.83	14.39	10

TABLE A-9

Carbon Coated Nuclepore Technique (Second 10 Samples)

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	Mass Conc. μg/l	χ^2(Total)	Significance Level of Uniformity %
3	4.98	2.30 - 7.66	0.76	10.4	6.90 - 13.9	0.32	15.4	1.08	12.86	10
7	4.60	1.14 - 8.06	0.47	6.48	2.86 - 10.1	0.24	11.1	0.71	17.61	2.5
13	5.34	2.31 - 8.37	0.61	7.26	3.98 - 10.5	0.15	12.6	0.76	25.45	0.1
17	6.30	3.06 - 9.54	1.01	6.50	2.89 - 10.1	0.14	12.8	1.15	17.71	2.5
23	6.96	4.96 - 8.96	1.07	8.02	3.55 - 12.5	0.36	15.0	1.43	8.44	10
27	2.55	1.24 - 3.86	0.23	2.55	0.82 - 4.28	0.04	5.1	0.27	16.03	5
33	6.49	3.52 - 9.46	0.39	3.82	1.96 - 5.68	0.06	10.3	0.45	12.09	10
37	9.03	5.24 - 12.8	0.61	5.42	3.01 - 7.83	0.10	14.5	0.71	13.71	10
43	8.78	5.86 - 11.7	1.00	5.31	2.76 - 7.86	0.48	14.1	1.48	9.39	10
47	7.32	4.92 - 9.72	0.98	4.43	1.96 - 6.90	0.10	11.8	1.08	12.32	10

TABLE A-10

Jaffé Washer Technique, 0.1μm Nuclepore

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
5	9.45	6.60 - 12.3	0.969	4.82	3.09 - 6.55	0.100	14.3	1.069	8.35	10
10	7.53	3.79 - 11.3	0.615	5.73	3.48 - 7.98	0.312	13.3	0.927	14.81	5
15	4.75	1.40 - 8.10	0.255	4.18	1.82 - 6.54	0.127	8.93	0.382	13.17	10
20	1.86	0.04 - 3.68	0.319	1.66	0 - 3.48	0.022	3.52	0.341	30.66	<0.1
25	6.36	4.06 - 8.66	0.517	5.05	3.40 - 6.70	0.076	11.4	0.593	9.22	10
30	2.40	0 - 4.82	0.449	2.40	0 - 5.04	0.029	4.80	0.478	53.41	<0.1
35	7.23	4.30 - 10.2	1.190	3.62	1.69 - 5.55	0.068	10.9	1.258	9.62	10
40	4.10	2.18 - 6.02	0.301	3.92	2.25 - 5.59	0.054	8.02	0.355	12.08	10
45	7.50	3.41 - 11.6	0.727	3.48	1.56 - 5.40	0.106	11.0	0.833	15.55	5
50	5.19	3.41 - 6.97	0.915	2.51	1.04 - 3.98	0.033	7.70	0.948	6.72	10

TABLE A-11

Jaffe Washer Technique, 0.45μm Millipore

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
5	2.82	0 - 6.48	0.479	1.30	0.27 - 2.33	0.028	4.12	0.507	35.76	<0.1
10	2.94	0.86 - 5.02	0.709	2.73	0.51 - 4.95	0.033	5.67	0.742	23.90	0.1
15	1.38	0.21 - 2.55	0.080	2.08	0 - 4.44	0.047	3.46	0.127	18.08	2.5
20	0.22	0 - 0.72	0.007	<0.22	-	-	0.22	0.007	8.29	10
25	1.46	0 - 3.54	0.100	1.46	0 - 3.41	0.083	2.92	0.183	38.02	<0.1
30	0.44	0 - 1.35	0.023	0.65	0 - 2.03	0.012	1.09	0.035	22.71	0.5
35	1.79	0 - 3.60	0.260	0.90	0 - 2.38	0.045	2.69	0.305	21.81	0.5
40	0.61	0 - 1.33	0.021	1.02	0 - 2.05	0.024	1.63	0.045	14.43	10
45	2.32	0 - 4.71	0.112	1.68	0 - 3.56	0.028	4.00	0.140	15.19	5
50	1.05	0 - 2.48	0.126	1.90	0.56 - 3.24	0.017	2.95	0.143	14.68	5

TABLE A-12

Condensation Washer Technique, 0.45μm Millipore

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	X^2(Total)	Significance Level of Uniformity %
5	4.50	1.75 - 7.25	0.941	4.30	1.67 - 6.93	0.399	8.80	1.340	14.74	5
10	2.86	0.21 - 5.51	0.195	4.40	1.48 - 7.32	0.108	7.26	0.303	24.17	0.1
15	4.18	1.55 - 6.81	0.815	5.23	0.85 - 9.61	0.129	9.41	0.944	40.51	<0.1
20	<0.19	-	-	0.19	0 - 0.64	0.002	0.19	0.002	51.14	<0.1
25	0.58	0 - 1.24	0.136	0.97	0 - 2.15	0.017	1.55	0.153	12.91	10
30	0.40	0 - 1.28	0.016	0.20	0 - 0.64	0.002	0.60	0.018	14.64	10
35	1.53	0 - 3.51	0.319	3.83	0.15 - 7.51	0.079	5.36	0.398	63.90	<0.1
40	1.37	0.43 - 2.31	0.113	2.94	0.95 - 4.93	0.041	4.31	0.154	12.92	10
45	0.41	0 - 1.02	0.644	0.41	0 - 1.34	0.007	0.82	0.651	11.10	10
50	3.87	1.68 - 6.06	1.440	2.37	0.07 - 4.67	0.029	6.24	1.469	19.93	1
20 *	3.53	0 - 7.08	0.733	2.23	0 - 5.00	0.036	5.76	0.769	51.14	<0.1
25 *	1.47	0 - 3.05	0.317	0.92	0.09 - 1.83	0.016	2.39	0.333	12.91	10
30 *	0.55	0 - 1.20	0.067	1.28	0 - 2.97	0.083	1.83	0.150	14.64	10
45 *	0.58	0 - 1.43	0.010	0.58	0 - 1.50	0.006	1.16	0.016	19.41	1

* Repeat Preparations

TABLE A-13

Jaffé Washer Technique, 0.22μm Millipore

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
2	1.96	0.18 - 3.74	0.766	2.40	0.43 - 4.37	0.018	4.36	0.784	42.65	<0.1
4	4.48	0 - 9.02	0.204	2.77	0 - 5.78	0.080	7.25	0.284	40.33	<0.1
6	0.68	0 - 1.75	0.237	1.14	0 - 2.49	0.030	1.82	0.267	7.29	10
8	1.13	0.21 - 2.05	0.096	2.82	0.24 - 5.40	0.044	3.95	0.140	15.78	5
10	4.18	1.38 - 6.98	0.397	5.28	2.45 - 8.11	0.231	9.46	0.628	9.13	10
12	6.95	3.53 - 10.4	0.364	8.18	4.17 - 12.2	0.162	15.1	0.526	15.06	5
14	6.40	3.55 - 9.25	0.441	6.19	2.54 - 9.84	0.113	12.6	0.554	25.74	0.1
16	1.58	0 - 3.55	0.188	1.58	0 - 3.38	0.020	3.16	0.208	32.93	<0.1
18	0.66	0.15 - 1.16	0.101	2.14	0 - 5.57	0.021	2.80	0.122	23.46	0.5
20	7.92	0.46 - 15.4	0.660	6.38	1.84 - 10.9	0.099	14.3	0.759	64.24	<0.1
6 *	1.93	0.64 - 3.22	0.172	0.86	0.07 - 1.65	0.003	2.79	0.175	7.29	10
16 *	3.45	0.31 - 6.59	0.426	3.85	0 - 7.76	0.076	7.30	0.502	32.93	<0.1
18 *	1.67	0.51 - 2.83	0.251	1.25	0 - 3.01	0.081	2.92	0.332	23.46	0.5

* Repeat Preparations

TABLE A-14

ORF Ashing Technique

Crocidolite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. µg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. µg/1	Mean Conc. x 10^6	Mass Conc. µg/1	χ^2(Total)	Significance Level of Uniformity %
3	5.16	2.71 - 7.61	0.654	4.17	1.54 - 6.80	0.155	9.33	0.809	11.58	10
7	3.81	2.00 - 5.62	1.36	2.80	0.95 - 4.65	0.072	6.61	1.43	14.08	10
13	2.77	0.58 - 4.96	0.263	3.96	0.37 - 7.55	0.130	6.73	0.393	29.02	<0.1
17	5.61	3.77 - 7.45	0.777	3.67	1.91 - 5.43	0.122	9.28	0.899	10.43	10
23	5.87	4.01 - 7.73	0.818	5.09	2.45 - 7.73	0.214	10.96	1.03	9.97	10
27	4.09	1.47 - 6.71	1.12	4.48	3.19 - 5.77	0.251	8.57	1.37	7.05	10
33	3.67	1.56 - 5.78	0.395	4.06	1.98 - 6.14	0.073	7.73	0.468	10.59	10
37	4.60	2.60 - 6.60	0.560	3.60	1.29 - 5.91	0.143	8.20	0.703	11.45	10
43	3.90	1.18 - 6.62	0.901	3.08	0.37 - 5.79	0.139	6.98	1.04	30.96	<0.1
47	4.44	1.32 - 7.56	0.761	3.55	1.55 - 5.55	0.136	7.99	0.897	11.45	10

TABLE A-15

Carbon Coated Nuclepore Technique (Initial 10 samples)

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. µg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. µg/1	Mean Conc. x 10^6	Mass Conc. µg/1	X^2(Total)	Significance Level of Uniformity %
5	10.3	5.70 - 14.9	17.5	4.49	1.67 - 7.31	0.30	14.8	17.8	6.25	10
10	12.5	8.10 - 16.9	26.8	4.94	1.55 - 8.33	0.20	17.4	27.0	14.24	10
15	12.5	8.60 - 16.4	18.0	3.72	0 - 7.48	0.33	16.2	18.3	12.69	10
20	12.3	7.60 - 17.0	13.1	5.21	3.02 - 7.40	0.76	17.5	13.9	7.99	10
25	14.3	6.60 - 22.0	22.6	7.13	5.32 - 8.94	0.28	21.4	22.9	9.35	10
30	14.0	10.1 - 17.9	4.49	9.31	5.97 - 12.7	0.35	23.3	4.84	5.83	10
35	24.1	11.3 - 36.9	7.66	9.00	2.80 - 15.2	0.21	33.1	7.87	31.86	<0.1
40	21.4	15.3 - 27.5	11.1	8.43	5.33 - 11.5	0.20	29.8	11.3	14.42	10
45	15.6	11.9 - 19.3	11.2	5.55	2.21 - 8.89	0.07	21.2	11.3	5.55	10
50	17.9	11.8 - 24.0	10.7	4.72	2.57 - 6.87	0.04	22.6	10.7	7.14	10

TABLE A-16

Carbon Coated Nuclepore Technique (Second 10 Samples)

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/l	Mean Conc. x 10^6	Mass Conc. μg/l	χ^2(Total)	Significance Level of Uniformity %
3	15.1	8.50 - 21.7	9.81	8.43	5.36 - 11.5	0.07	23.5	9.88	11.18	10
7	17.4	10.3 - 24.5	3.75	12.4	6.90 - 17.9	0.16	29.8	3.91	11.38	10
13	23.7	19.9 - 27.5	3.75	10.2	7.20 - 13.2	0.08	33.9	3.83	11.34	10
17	17.8	14.2 - 21.4	2.88	9.52	5.99 - 13.1	0.08	27.3	2.96	13.35	10
23	16.7	12.7 - 20.7	14.6	10.8	6.00 - 15.6	0.10	27.5	14.7	16.14	5
27	11.9	8.10 - 15.7	6.43	12.5	7.30 - 17.7	0.18	24.4	6.61	8.23	10
33	16.7	12.8 - 20.6	5.19	12.7	8.10 - 17.3	0.12	29.4	5.31	4.18	10
37	13.2	7.20 - 19.2	8.82	12.1	8.60 - 15.6	0.12	25.3	8.94	15.42	5
43	18.0	12.3 - 23.7	3.85	13.4	9.40 - 17.4	0.15	31.4	4.00	3.76	10
47	19.7	11.8 - 27.6	3.64	9.55	5.24 - 13.9	0.08	29.3	3.72	27.64	0.1

TABLE A-17

Jaffe Washer Technique, 0.1 μm Nuclepore

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2 (Total)	Significance Level of Uniformity %
5	14.7	11.0 - 18.4	6.75	17.0	10.2 - 23.8	0.220	31.7	6.97	11.85	10
10	27.9	22.7 - 33.1	4.23	14.0	7.90 - 20.1	0.144	41.9	4.37	6.49	10
15	30.3	20.6 - 40.0	8.13	14.8	10.1 - 19.5	0.105	45.1	8.24	13.05	10
20	31.0	22.8 - 39.2	21.7	16.5	10.1 - 22.9	0.177	47.5	21.88	10.02	10
25	26.3	19.7 - 32.9	3.69	13.4	8.40 - 18.4	0.111	39.7	3.80	10.96	10
30	19.4	16.4 - 22.4	29.5	15.7	9.50 - 21.9	0.240	35.1	29.7	6.93	10
35	29.3	20.2 - 38.4	6.66	14.6	10.2 - 19.0	0.127	43.9	6.79	5.70	10
40	22.1	11.6 - 32.6	7.97	13.7	7.80 - 19.6	0.116	35.8	8.09	28.39	<0.1
45	28.3	24.0 - 32.6	5.44	17.9	13.4 - 22.4	0.110	46.2	5.55	6.37	10
50	24.3	18.1 - 30.5	8.14	16.3	7.90 - 24.7	0.127	40.6	8.27	7.92	10

TABLE A-18

Jaffe Washer Technique, 0.45μm Millipore

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	X^2(Total)	Significance Level of Uniformity %
5	7.74	0.58 - 14.9	8.86	4.15	0.14 - 8.16	0.04	11.9	8.90	19.90	1
10	5.57	0 - 12.1	2.45	4.11	0.86 - 7.36	0.06	9.68	2.51	32.17	<0.1
15	5.49	1.50 - 9.48	2.96	1.16	0 - 2.64	0.01	6.65	2.97	12.46	10
20	5.14	1.05 - 9.23	1.29	3.02	1.36 - 4.68	0.07	8.16	1.36	7.89	10
25	3.22	0.56 - 5.88	1.42	1.47	0.13 - 2.81	0.01	4.69	1.43	15.14	5
30	6.74	1.36 - 12.1	7.55	2.64	0.73 - 4.55	0.06	9.38	7.61	22.50	0.5
35	6.16	2.46 - 9.86	3.59	2.93	1.17 - 4.69	0.02	9.09	3.61	15.50	5
40	3.58	0.74 - 6.42	3.23	1.93	0.11 - 3.75	0.02	5.51	3.25	15.11	5
45	3.96	2.14 - 5.78	5.32	1.52	0 - 3.10	0.14	5.48	5.46	7.20	10
50	4.77	2.42 - 7.12	1.55	3.71	0.49 - 6.93	0.05	8.48	1.60	15.66	5

TABLE A-19

Condensation Washer Technique, 0.45μm Millipore

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
5	8.39	3.49 - 13.3	12.9	4.84	2.48 - 7.20	0.03	13.2	12.9	10.11	10
10	9.68	5.59 - 13.8	4.15	6.08	3.58 - 8.58	0.05	15.8	4.20	6.94	10
15	1.87	0 - 4.05	2.41	2.80	0.95 - 4.65	0.03	4.67	2.44	17.98	2.5
20	3.91	0 - 8.41	3.18	3.91	1.33 - 6.49	0.03	7.82	3.21	35.29	<0.1
25	2.98	0.46 - 5.50	0.28	1.99	0 - 4.01	0.02	4.97	0.30	15.20	5
30	7.04	0 - 14.4	8.53	3.67	0.35 - 6.99	0.03	10.7	8.56	46.96	<0.1
35	3.46	0.31 - 6.61	0.72	2.13	0.44 - 3.82	0.04	5.59	0.76	29.68	<0.1
40	2.87	0.58 - 5.16	0.89	1.43	0.03 - 2.83	0.02	4.30	0.91	9.50	10
45	8.87	5.91 - 11.8	11.8	3.67	1.01 - 6.33	0.03	12.5	11.8	7.23	10
50	5.39	1.35 - 9.43	7.23	4.25	1.02 - 7.48	0.06	9.64	7.29	15.19	5

TABLE A-20

Jaffe Washer Technique, 0.22μm Millipore

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
2	4.73	1.41 - 8.05	20.0	3.68	0.98 - 6.38	0.02	8.41	20.0	22.88	0.5
4	11.6	7.00 - 16.2	5.83	4.49	1.69 - 7.29	0.05	16.1	5.88	15.57	5
6	6.37	3.60 - 9.14	6.29	3.76	1.61 - 5.91	0.02	10.1	6.31	7.01	10
8	11.2	5.10 - 17.3	14.1	5.32	2.95 - 7.69	0.02	16.5	14.1	17.85	2.5
10	10.3	5.10 - 15.5	5.49	9.51	5.07 - 14.0	0.05	19.8	5.54	13.59	10
12	4.36	1.63 - 7.09	5.79	2.18	0.11 - 4.25	0.01	6.54	5.80	24.86	0.1
14	5.62	2.60 - 8.64	2.05	4.02	2.18 - 5.86	0.02	9.64	2.07	9.53	10
16	9.00	5.73 - 12.3	16.0	4.91	2.12 - 7.70	0.05	13.9	16.1	7.93	10
18	7.15	2.28 - 12.0	5.61	5.30	2.39 - 8.21	0.04	12.5	5.65	12.02	10
20	6.18	1.73 - 10.6	1.45	5.90	0 - 12.7	0.03	12.1	1.48	42.01	<0.1

TABLE A-21

ORF Ashing Technique

Taconite

Filter No.	Confirmed Amphibole Fibers			Probable Amphibole Fibers			Total Conc.		Grid Distribution	
	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	95% Confidence Interval x 10^6	Mass Conc. μg/1	Mean Conc. x 10^6	Mass Conc. μg/1	χ^2(Total)	Significance Level of Uniformity %
2	22.6	15.8 - 29.4	9.66	12.1	6.20 - 18.0	0.23	34.7	9.89	15.00	5
8	21.3	11.2 - 31.4	8.76	4.11	0.79 - 7.43	0.06	25.4	8.82	12.71	10
12	26.3	20.3 - 32.3	26.6	12.6	7.90 - 17.3	0.15	38.9	26.8	5.33	10
18	23.7	18.8 - 28.6	10.6	13.1	9.30 - 16.9	0.23	36.8	10.8	7.13	10
22	22.6	17.9 - 27.3	18.3	10.2	7.00 - 13.4	0.13	32.8	18.4	2.77	95
28	20.2	14.2 - 26.2	13.6	11.8	8.20 - 15.4	0.27	32.0	13.9	4.05	10
32	25.0	13.5 - 36.5	17.8	10.5	6.20 - 14.8	0.17	35.5	18.0	16.17	5
38	23.6	14.0 - 33.2	6.00	8.55	4.78 - 12.9	0.19	32.5	6.19	12.11	10
42	18.5	13.1 - 23.9	14.6	10.5	5.30 - 15.7	0.16	29.0	14.8	13.05	10
48	19.6	14.0 - 25.2	17.6	7.70	4.59 - 10.8	0.10	27.3	17.7	8.71	10

TABLE A-22

ORF Ashing Technique: Chrysotile *

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration μg/l	Grid Distribution	
				x^2	Significance Level of Uniformity %
3	24.0	18.2 - 29.8	0.236	16.04	5
7	36.4	30.1 - 42.7	0.397	10.20	10
13	29.2	22.7 - 35.7	0.365	13.35	10
17	50.9	44.8 - 57.0	0.461	5.98	10
23	35.9	29.3 - 42.5	0.304	12.97	10
27	30.5	24.4 - 36.6	0.221	9.94	10
33	46.0	40.3 - 51.7	0.327	6.73	10
37	41.7	36.2 - 47.2	0.336	5.60	10
43	42.2	33.9 - 50.5	0.309	11.06	10
47	43.2	36.7 - 49.7	0.291	10.93	10

* Concentrations include contamination contribution

TABLE A-23

Chrysotile Contamination in Millipore Filters *

Filter No.	Mean Fiber Concentration x 10^6	95% Confidence Interval x 10^6	Mass Concentration µg/l	Grid Distribution	
				χ^2	Significance Level of Uniformity %
Croc. 3	13.0	9.15 - 16.8	0.121	12.01	10
Croc. 7	8.70	4.43 - 13.0	0.044	17.10	2.5
Croc.13	5.64	4.37 - 6.91	0.044	3.96	10
Croc.17	10.4	7.58 - 13.3	0.055	9.15	10
Croc.23	19.8	14.6 - 25.1	0.267	15.47	5
Croc.27	9.68	5.18 - 14.2	0.067	31.24	< 0.1
Croc.33	20.0	13.4 - 26.6	0.095	17.45	2.5
Croc.37	15.9	10.7 - 21.2	0.098	26.54	0.1
Croc.43	8.78	5.25 - 12.3	0.042	15.33	5
Croc.47	15.3	10.4 - 20.3	0.080	19.71	1

* Results obtained by counting chrysotile on filters loaded with crocidolite

TABLE A-24

Effects of Ultrasonification

Aqueous Chrysotile Dispersion, 0.05 watt/ml

Operator	Exposure Time Seconds	Mean Crocidolite Concentration x 10^6 fibers/l	95% Confidence Interval x 10^6 fibers/l	Mass Concentration μg/l	Grid Distribution	
					X^2	Significance Level of Uniformity %
A	0	46.3	32.8 –- 59.8	2.15	16.59	5
	15	36.4	28.3 - 44.5	2.64	7.36	10
	60	52.9	39.2 - 66.6	3.71	17.27	2.5
	300	53.8	40.8 - 66.8	3.32	12.80	10
B	0	73.3	56.3 - 90.3	3.20	19.13	1
	15	67.4	56.3 - 78.5	3.48	9.48	10
	60	82.6	57.2 - 108.0	4.41	41.11	<0.1
	300	111.0	80.0 - 142.0	5.32	36.13	<0.1

TABLE A-25

Effects of Ultrasonification

Aqueous Chrysotile Dispersion, 0.5 watt/ml

Operator	Exposure Time Seconds	Mean Crocidolite Concentration x 10^6 fibers/l	95% Confidence Interval x 10^6 fibers/l	Mass Concentration μg/l	Grid Distribution	
					X^2	Significance Level of Uniformity %
A	0	42.2	29.1 - 55.3	1.90	21.02	1
	15	56.9	44.6 - 69.2	4.02	18.16	2.5
	60	47.8	32.1 - 63.5	3.91	18.03	2.5
	300	79.3	62.9 - 95.7	3.66	14.53	10
B	0	59.8	41.4 - 78.2	2.87	35.17	<0.1
	15	79.9	55.3 - 104.5	5.76	23.93	0.1
	60	89.7	77.2 - 102.2	5.35	39.88	<0.1
	300	92.6	77.9 - 107.3	3.17	9.04	10

TABLE A-26

Effects of Ultrasonification

Aqueous Crocidolite Dispersion, 0.05 watt/ml

Operator	Exposure Time Seconds	Mean Crocidolite Concentration x 10^6 fibers/1	95% Confidence Interval x 10^6 fibers/1	Mass Concentration µg/1	Grid Distribution	
					X^2	Significance Level of Uniformity %
A	0	50.3	36.3 - 64.3	6.61	20.29	1
	15	58.7	43.1 - 74.3	8.31	12.36	10
	60	64.8	48.8 - 80.8	10.0	16.29	5
	300	60.3	47.6 - 73.0	10.2	6.19	10
B	0	53.1	34.5 - 71.7	7.72	34.77	<0.1
	15	57.3	40.6 - 74.0	9.29	19.82	1
	60	59.2	37.9 - 80.5	7.37	23.20	0.5
	300	59.6	45.2 - 74.0	7.54	18.28	2.5

TABLE A-27

Effects of Ultrasonification

Aqueous Crocidolite Dispersion, 0.5 watt/ml

Operator	Exposure Time Seconds	Mean Crocidolite Concentration x 10^6 fibers/l	95% Confidence Interval x 10^6 fibers/l	Mass Concentration µg/l	Grid Distribution	
					χ^2	Significance Level of Uniformity %
A	0	74.7	51.2 - 98.2	10.6	22.02	0.5
	15 *	76.0	5.3 - 146.7	7.72	201.98	<0.1
	60	93.9	67.3 - 120.5	9.03	16.01	5
	300	104.0	92.0 - 116.0	16.7	10.21	10
B	0	73.6	49.0 - 98.2	9.07	27.13	0.1
	15	77.9	53.1 - 102.7	9.38	48.30	<0.1
	60	86.9	60.7 - 113.1	10.5	20.11	1
	300	58.0	37.8 - 78.2	9.70	21.05	1

* Filter reported non-uniform by operator